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Improved production of protease-resistant phytase by *Aspergillus oryzae* and its applicability in the hydrolysis of insoluble phytates

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Abstract Among three hundred isolates of filamentous fungi, *Aspergillus oryzae* SBS50 secreted higher phytase activity at pH 5.0, 35 °C and 200 rpm after 96 h of fermentation. Starch and beef extract supported the highest phytase production than other carbon and nitrogen sources. A nine-fold improvement in phytase production was achieved due to optimization. Supplementation of the medium with inorganic phosphate repressed the enzyme synthesis. Among surfactants tested, Tween 80 increased fungal growth and phytase production, which further resulted in 5.4-fold enhancement in phytase production. The phytase activity was not much affected by proteases treatment. The enzyme resulted in the efficient hydrolysis of insoluble phytate complexes (metal- and protein–phytates) in a time dependent manner. Furthermore, the hydrolysis of insoluble phytates was also supported by scanning electron microscopy. The enzyme, being resistant to trypsin and pepsin, and able to hydrolyze insoluble phytates, can find an application in the animal food/feed industry for improving nutritional quality and also in combating environmental phosphorus pollution and plant growth promotion.

Keywords Phytase · Submerged fermentation · Insoluble phytates · Protease-resistant · Scanning electron microscopy

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Introduction

Phosphorus is an essential element for the growth of all living organisms, therefore, food and feed must be supplemented with inorganic phosphorus in order to meet their daily requirements [17, 23, 26]. One-third of phosphorus in plant derived food is present as a digestible inorganic form and two-thirds as organic phosphorus in the form of phytin, which is a mixture of calcium–magnesium salts of phytic acid [9, 14, 17]. This phytic phosphorus cannot be digested by monogastric animals due to the lack of adequate levels of phytases in their digestive tract [17]. The unabsorbed phosphorus passes into the environment by faeces and causes environmental pollution due to eutrophication [14, 23, 26]. Reduction or elimination of inorganic phosphate supplementation to animal feed reduces phosphorus in the manure by about 33 %, thus cutting the pollution burden by one-third [14]. This type of pollution can be reduced to some extent with the help of phytate degrading enzymes, i.e., phytases [14, 17, 27].

Phytases are mainly produced by microorganisms such as fungi, bacteria and yeasts [14, 17, 23, 26, 27]. Most of the scientific work has been carried out on microbial phytases, especially on those originating from filamentous fungi such as *Aspergillus niger* van Teighem [24], *Thermoascus aurantiacus* [10], *A. niger* CFR 335 [6], *Sporotrichum thermophile* [15, 16], *A. niger* NCIM 563 [1, 12] and *A. niger* st-6 [19]. The use of filamentous fungi for the production of commercially important metabolites has increased rapidly over the past half century and the production of enzymes in submerged fermentation has long been established [15, 16, 19]. However, there are very few reports on production of a protease-resistant phytase from a fungal source [3, 15] as well as applicability of phytase in hydrolysis of insoluble phytates [18, 19, 22]. Interaction

with positively charged dietary proteins leads to the formation of phytate–protein complexes, which decrease their accessibility to protease and, therefore, reducing protein digestion [22]. In the present investigation, we have reported the production of a protease-resistant phytase by *A. oryzae* SBS50 in submerged fermentation and its applicability in the hydrolysis of insoluble phytates (metal-phytates and protein-phytates). To the best of our knowledge, this is the first report in the area of phytase research revealing the hydrolysis of insoluble phytates by scanning electron microscopy.

Materials and methods

Source of strain and culture conditions

Aspergillus oryzae SBS50 was isolated from a soil sample collected from Rohtak, Haryana (India) and was routinely grown on PDA medium. The cultures were maintained on PDA slants at 4 °C and in glycerol at −20 °C.

Molecular identification

The mycelial biomass was suspended in the extraction buffer (50 mM Tris–HCl, pH 8; 50 mM EDTA; 2 % SDS; 1 % Triton X 100) along with glass beads (diam. 0.5 mm) [1:1.6:0.6] and homogenized for 2 min. in a bead beater (20 s pulses with intermittent cooling in ice). After centrifugation, the cell-free supernatant was used as the source of crude genomic DNA. The genomic DNA was purified using phenol, chloroform and isoamyl alcohol [8]. The DNA was precipitated with isopropanol and washed with ethanol and resuspended in milliQ water and the purified genomic DNA was used as template for PCR amplification of ITS sequences using universal primers ITS1 (TCCGTAG-GTGAACCTGCGG) and ITS 4 (TCCTCCGCTTATTGATATGC). The PCR was performed in a 0.2 ml tube with a total volume of 50 µl reaction mixture containing 0.8 µM forward and reverse primer, 200 µM dNTPs, 2.5 U of Taq DNA polymerase, 2 µl of template DNA in 5 µl PCR buffer (2 mM $MgCl_2$). Gradient PCR was carried out in a thermocycler (C1000 Thermal Cycler, BioRad) with the program: (1) initial denaturation—95 °C for 5 min; (2) 30 cycles—94°C for 30 s; 56–62 °C for 30 s; 72 °C for 1.5 min; (3) final extension at 72 °C for 10 min. The PCR product was electrophoresed on 0.8 % agarose gel and analyzed after staining with ethidium bromide. The amplicon of 545 bp was excised and purified with a DNA extraction kit (Geneaid) and sequenced. The sequence was used for identifying the fungus with the help of the BLASTn program (www.ncbi.nlm.nih.gov/BLAST) and multiple-sequence alignments using ClustalW program.

Measurement of phytase activity and fungal biomass estimation

Phytase was assayed by determining the amount of phosphate liberated using phytate as the substrate [15, 16]. The reaction mixture consisted of 0.5 ml substrate (2 mM) prepared in 0.1 M acetate buffer (pH 5.0) and 0.5 ml of crude enzyme. After incubation at 50 °C, the reaction was stopped by adding 1 ml of 10 % trichloroacetic acid. The amount of free phosphate released was determined according to Fiske and Subbarow [4]. One unit of phytase is defined as the amount of enzyme required to liberate 1 µmole of inorganic phosphate per min under the assay conditions using KH_2PO_4 as the standard.

Fungal biomass was estimated gravimetrically by filtering the culture broth through a pre-weighed dry Whatman No. 1 filter paper circles. The mycelium was thoroughly washed with double distilled water and dried at 80 °C to constant weight.

Optimization of phytase production by *A. oryzae* in submerged fermentation

Preparation of spore suspension of A. oryzae SBS50

Spore suspension of the fungus was prepared from 72 h old PDA slants by adding 25 ml of normal saline containing Tween 80 (0.1 % v/v) followed by shaking and filtration. The viable spore count of the spore suspension was carried out by using a suitable dilution followed by counting the number of colonies. Spore suspension with a viable spore count of 4×10^7 (CFU/ml) was used for inoculation.

Selection of medium

Three different media viz. medium 1 [7], medium 2 [24] and medium 3 were tested for phytase production by *A. oryzae* (Table 1). Initial pH of each medium was kept at pH 5.0. The liquid medium in 250 ml flasks (50 ml) was incubated at 30 °C and 200 rpm after inoculation. The cell-free culture filtrate was analyzed for phytase activity.

Effect of cultural parameters on phytase production

Phytase production by *A. oryzae* SBS50 was optimized in submerged fermentation by the one variable at a time approach, changing one variable while keeping all others at their constant level. The medium 2 [24] was selected for the optimization of phytase production as it supported high enzyme titers as compared to others. Fifty ml medium in a 250 ml Erlenmeyer flask was sterilized and inoculated with 1 ml spore suspension containing 4×10^7 spores prepared from a 3-day-old slant. Phytase production was studied at

Table 1 Composition of different media used for the cultivation of *Aspergillus oryzae* SBS50

Components	Quantity (g/l)
Medium 1 [7]	
Glucose	15
Ammonium sulphate	5
Magnesium sulphate	5
Manganese sulphate	0.1
Potassium chloride	0.5
Ferrous sulphate	0.1
Medium 2 [24]	
Glucose	30
Starch	10
Ammonium sulphate	5
Magnesium sulphate	0.5
Potassium chloride	0.5
Ferrous sulphate	0.1
Medium 3 (peptone dextrose medium)	
Peptone	10
Dextrose	40

different pH values ranging from 3.0 to 8.0. To determine the optimal incubation temperature for phytase production, inoculated flasks were incubated at different temperatures (25–40 °C) for three days with constant shaking in an incubator shaker. After incubation for 72 h, the culture filtrate was analyzed for phytase activity.

Different carbon sources like glucose, starch, sucrose, wheat bran and cane molasses were studied to observe their effect on enzyme production. Phytase production was also tested using various inorganic (ammonium nitrate, sodium nitrate, ammonium sulfate) and organic (peptone, yeast extract, beef extract, tryptone, and urea) nitrogen sources at 0.5 % concentration. Effect of different surfactants [Tween 80, Tween 60, SDS (sodium dodecyl sulphate), CTAB (Cetyl trimethylammonium bromide) and Triton X 100] on phytase production was also studied. The effect of different concentrations of inorganic phosphate in the form of KH_2PO_4 was assessed on phytase production.

Preparation and hydrolysis of insoluble metal–phytates and protein–phytates

In order to study the hydrolysis of metal phytates, 100 mM stock solutions of metals ions (Ca^{2+}, Co^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+}) were prepared by dissolving $CaCl_2{\cdot}2H_2O$, $COCl_2{\cdot}2H_2O$, $FeSO_4{\cdot}7H_2O$, $MnCl_2{\cdot}4H_2O$, $MgSO_4{\cdot}7H_2O$ and $ZnCl_2{\cdot}7H_2O$ in distilled water [18, 20]. Equal volumes (0.8 ml each) of 100 mM salt solution and 10 mM sodium phytate were mixed and incubated overnight at 4 °C. The precipitated salts were centrifuged at 2,000 rpm at 4 °C for 1 min. The supernatant was decanted off and the precipitated salts were washed thrice with distilled water and finally suspended in 0.5 ml of 0.1 M Na-acetate buffer (pH 5.0) [18, 20]. The hydrolysis reactions were initiated by incubating each phytate salt with 1U of phytase at 25 and 50 °C. Aliquots of the mixture were taken at desired intervals. The salts were pelleted by centrifugation and the amount of inorganic phosphate in the supernatant was determined as described earlier. The substrate and enzyme controls were also run simultaneously and values were deducted from the test value.

The phytate protein complex was prepared by mixing sodium phytate (3 mM) and lysozyme (2.5 mM) in distilled water as described by Tran et al. [22]. Protein–phytate complex was centrifuged and suspended in phytase solution (2 U). The reaction was carried out at 30 °C. The absorbance at 600 nm was monitored in a UV–Vis spectrophotometer. Simultaneously, a 10 ml reaction mixture was incubated at 30 °C and aliquots of the mixture were taken at different time intervals. The samples were analyzed for inorganic phosphorus estimation as described earlier.

Effect of proteases on phytase activity

Enzyme was incubated for 30–120 min at 37 °C in the presence of 1 % (w/v) trypsin and pepsin, and used in phytase assays to assess the effect of proteases on phytase activity [15].

All experiments were carried out in triplicates and their average values have been presented.

Scanning electron microscopy of the insoluble phytates

Insoluble metal–phytates and protein–phytate complexes were treated with phytase and samples were withdrawn at definite intervals as described above. After incubation of 60 and 120 min, the mixture was centrifuged at 11,000 rpm for 1 h and the pellet was collected to visualize the effects of crude enzyme by scanning electron microscopy. Control comprised of each material in same reaction mixture except enzyme. For primary fixation, samples were placed in 2.5 % glutaraldehyde solution for 2 h. After that, dehydration was carried out gradually (25, 50, 70, 95, and 100 %) with ethanol solution, and was air dried. The samples were metalized with gold and were analyzed in a scanning electron microscope (JEOL, JSM-6510).

Results and discussion

Phytates have been considered as a threat in the diets of non-ruminants due to its anti-nutritional properties [14, 17, 23, 26, 27]. The enzymatic reduction of these phytates is preferred in

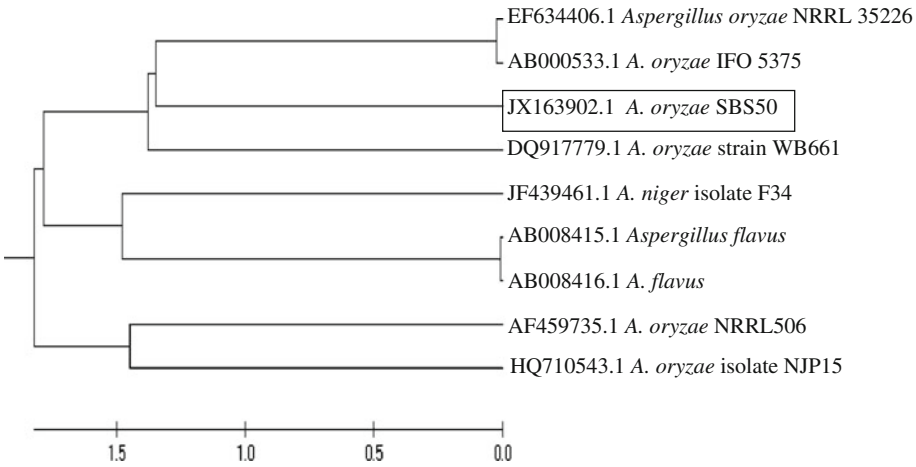

Fig. 1 Phylogenetic tree showing the relationship of ITS sequences of *Aspergillus oryzae* SBS50 with other fungi

the food industry because of the lack of undesirable side reactions and products with minimal or no loss in nutritional quality. However, there are still limited sources of phytase suited for all food application [14]. Thus, screening for ideal phytase with more improved properties and engineering phytases in order to optimize their catalytic and stability features are of research interest [5, 14]. We have isolated 300 mesophilic fungi and screened for phytase production. Isolate SBS50 showed higher phytase production as compared to other fungi. Among different media studied, medium 2 [24] supported a high enzyme production (333 U/l) as compared to other media by the selected isolate. Therefore, this medium was selected for further optimization.

Molecular identification of the potent phytase-producing mould

Among 300 fungal cultures from 72 soil samples collected from different geographical regions of India, *A. oryzae* SBS50 was selected as potent phytase producer. The fungus was identified based on morphological features and followed by ITS sequencing. The ITS region of the isolated SBS50 strain was amplified by PCR as described in the materials and methods section. The amplification size was 545 bp which showed 99 % identity with those of the *A. oryzae* strain NRRL35226 (Gen Bank accession no. EF634406.1), *A. oryzae* strain IFO5375 (Gen Bank accession no. AB000533.1) and the *A. oryzae* strain WB661 (Gen Bank accession no. DQ917779.1). Therefore, strain SBS50 was identified as *A. oryzae* under a Gen Bank accession no. JX163902.1 (Fig. 1).

Effect of cultural parameters on phytase production

Effect of pH and temperature on enzyme production

The medium pH is one of the regulatory parameters during microbial fermentation. Maximum phytase production was

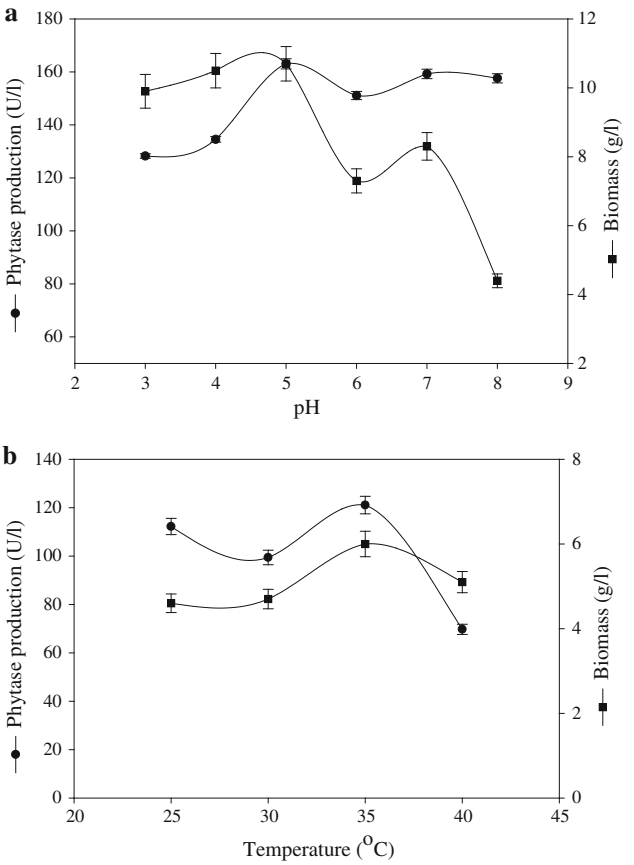

Fig. 2 **a** Effect of pH on phytase production by *A. oryzae* SBS50 in submerged fermentation, **b** effect of temperature on phytase production by *A. oryzae* SBS50

observed between pH 4 and 5, with the highest at pH 5 (163.55 U/l) (Fig. 2a). Several investigators have reported that acidic pH favours the phytase production by filamentous fungi [13, 14, 17, 23, 26, 27]. Similarly, *A. ficuum* NCIM563 [1] and *S. thermophile* [16] produced phytase optimally at pH 5.5 and 5.0, respectively. *Aspergillus oryzae* SBS50 secreted phytase optimally at 35 °C

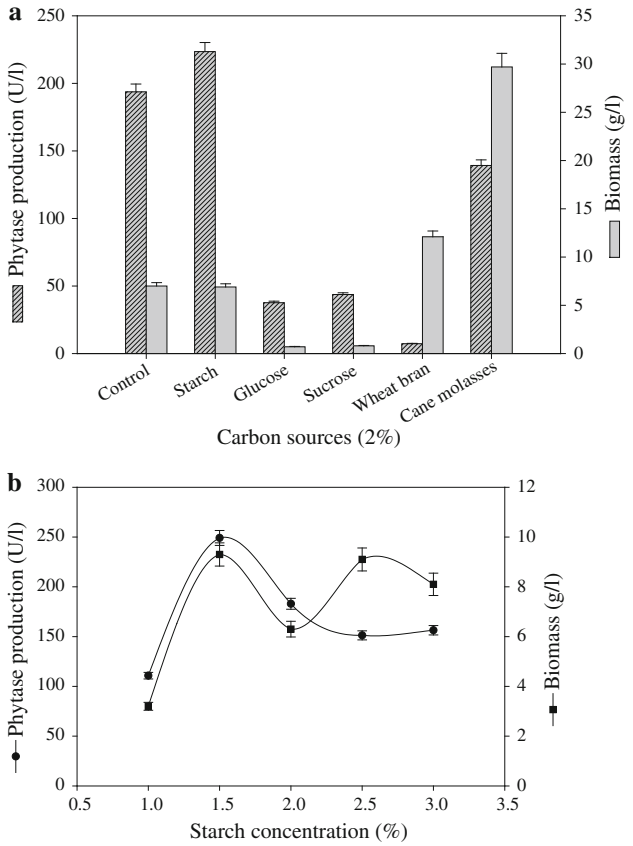

Fig. 3 **a** Effect of carbon sources on phytase production by *A. oryzae* SBS50 in submerged fermentation, **b** effect of different concentrations of starch on phytase production

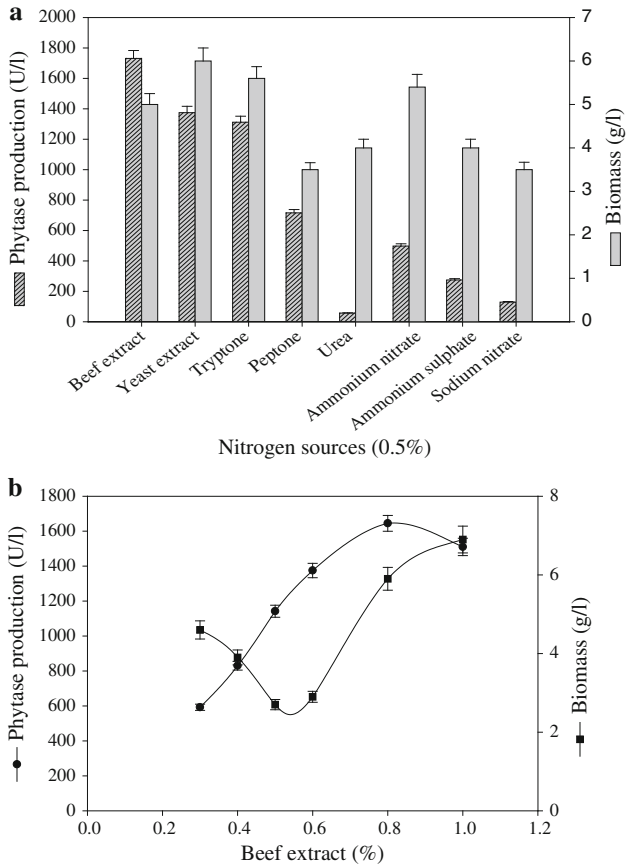

Fig. 4 **a** Effect of different organic and inorganic nitrogen sources on phytase production, **b** effect of different concentrations of beef extract on phytase production

(Fig. 2b), which is also the optimum temperature for its growth. Other filamentous fungi like *A. niger* NCIM 563 [1], *R. chinensis* [21] and a thermophilic mould *S. thermophile* [15, 16] also produced phytases at temperatures optimal for their growth.

Effect of Carbon sources

Carbon sources are some of the essential constituents of the microbial fermentation medium, which affects the overall cellular growth and metabolism. Among the different carbon sources, starch at a concentration of 1.5 % showed the highest phytase production (249.07 U/l) followed by a decline in phytase production with increasing concentrations (Fig. 3a, b). The enzyme titre was the lowest in the case of wheat bran. Similar reports of phytase production from various *Aspergillus* spp [13, 24] and *S. thermophile* [16] indicated use of starch as a carbon source. The maximum enzyme production by *A. niger* van Teigham was obtained when starch was used as carbon source, followed by a combination of glucose and starch [24]. In contrast, glucose was utilized as carbon source by *A. niger* NCIM 563 [1, 12] and *P. anomala* [25] for phytase production.

Effect of nitrogen sources

Phytase production was studied in the presence of various organic and inorganic nitrogen sources. Organic nitrogen sources such as peptone, tryptone, yeast extract and beef extract resulted in higher enzyme titers as compared to inorganic sources (Fig. 4a, b). Among the organic nitrogen sources, 0.8 % beef extract showed maximum phytase production (1,731.15 U/l) as reported in *P. anomala* [25]. In contrast, *A. niger* NCIM563 [1] and *S. thermophile* [15, 16] produced maximum phytase using sodium nitrate and ammonium sulphate, respectively.

Effect of incubation period on phytase production

Phytase activity was highest (2,881.60 U/l) after four days of incubation and declined with the increase in incubation time (Fig. 5). There was very much less enzyme activity on the eighth day in the culture supernatant. It was found that enzyme production took place during the period of logarithmic phase. The fungal biomass also decreased on prolonged incubation. The reason may be cell lyses due to the

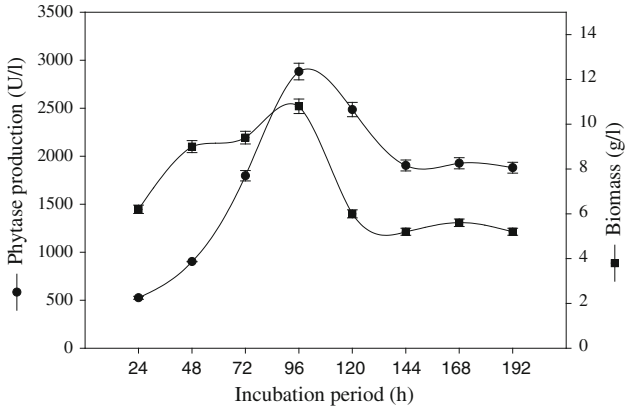

Fig. 5 Effect of incubation period on phytase production by *A. oryzae* SBS50

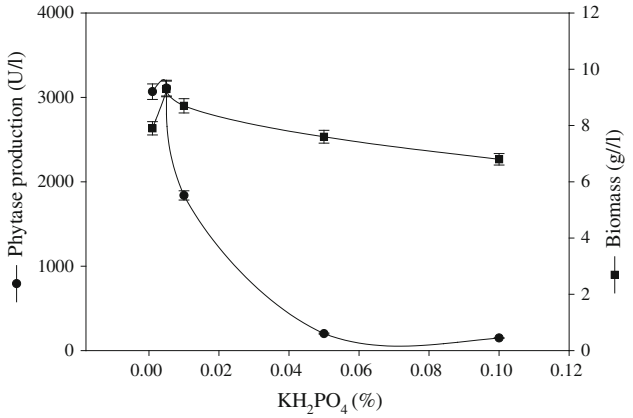

Fig. 6 Effect of different concentrations of inorganic phosphate on phytase production

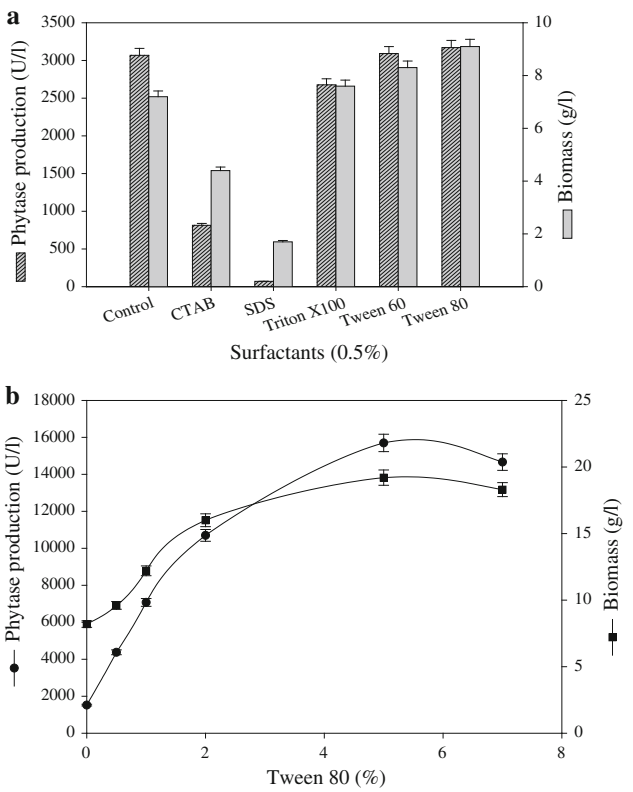

Fig. 7 **a** Effect of supplementation of different surfactants on phytase production, **b** effect of different concentrations of Tween 80 on phytase production

exhaustion of nutrients [11]. The best incubation period for phytase production in *A. niger* NCIM 563 [1] and *S. thermophile* [16] was reported at 24 and 96 h, respectively.

Effect of inorganic phosphate on phytase production

Phytase production decreased with the supplementation of inorganic phosphate in the medium even at low concentration (Fig. 6), which could be due to the repression of enzyme synthesis, as high phosphate levels are known to repress the synthesis of acid phosphatases and phytases [15, 16, 24, 26]. Nampoothiri et al. [10] have also recorded enhanced phytase secretion by *T. aurantiacus* at low level of inorganic phosphate followed by reduction at higher levels.

Effect of surfactants on phytase production

Membrane proteins are tightly bound to the lipids by a combination of hydrophobic and ionic bonds, and therefore, more drastic procedures have to be applied for breaking these bonds, such as the use of detergents and aqueous organic solvents to enhance the secretion of microbial enzymes [10, 16]. The permeabilization of membrane facilitates the secretion of proteins. Surfactants are known to affect the growth and enzyme production in filamentous fungi [10], and therefore, have been used in biotechnology for improving the yield of a number of enzymes produced by fermentation. Among various surfactants tested, Tween 80 showed high phytase production by *A. oryzae* (3,170.37 U/l) as compared to other surfactants (Fig. 7a). The SDS negatively affected the growth as well as phytase production by *A. oryzae*. It was found that 5 % Tween 80 (v/v) showed a 5.4-fold enhancement in phytase production (15,700.0 U/l) followed by a decline at higher concentrations. Biomass of the mould was also increased with increasing concentrations of Tween 80. Similarly, Tween 80 enhanced phytase production in *A. niger* NCIM 563 [1, 2], *S. thermophile* [16] and *T. aurantiacus* [10]. In contrast, addition of Tween 20 enhanced phytase production by *A. niger* CFR335 [6]. The productivity of phytase was also enhanced from 4.63 to 163.54 U/l/h as a result of optimization (Table 2).

Table 2 Comparison of phytase production under optimized and unoptimized culture conditions

Condition	Phytase production (U/l)	Fold improvement	Fermentation time (h)	Productivity (U/l/h)
Unoptimized	333.0	1	72	4.63
Optimized with out Tween-80	2,881.6	8.65	96	30.02
Optimized with Tween-80	15,700.0	5.45	96	163.54

Effect of proteases on phytase of *A. oryzae*

Phytase is used as an important animal feed supplement, and, therefore, it should resist the action of trypsin and pepsin that are present in animal digestive tracts. The enzyme activity was not affected when treated with trypsin and pepsin (Table 3). Treatment with trypsin resulted in 27 and 38 % reduction only in phytase activity after 60 and 120 min, respectively. However, there was no major effect on enzyme activity observed with pepsin. Similar results were observed for the phytase of *S. thermophile* BJTLR50 [15] and *A. oryzae* AK9 [3].

Hydrolysis of insoluble phytates

Phosphorus deficiency in soils is a major constraint for agricultural production worldwide. In soil, phytic acid is either adsorbed to clays or precipitated as insoluble salts of iron and aluminum in acidic soils or as insoluble calcium salts in alkaline soils [20]. Therefore, a phytase with high activity on phytates has a demand for soil amendment and plant growth promotion [18]. Plants fail to utilize the insoluble phytates directly, and, therefore, these phytates should be hydrolyzed by phytases before their assimilation by plants. Phytase of *A. oryzae* was able to hydrolyze various insoluble phytates to a varied extent. The hydrolysis rate of insoluble phytates was higher at 50 °C (Fig. 8b) than that at 25 °C (Fig. 8a) as the enzyme displayed its optimal activity at 50 °C. It released inorganic phosphate from Ca^{2+}, Fe^{2+}, and Co^{2+} phytates more efficiently than those of Fe^{3+}, Mg^{2+}, Mn^{2+} and Zn^{2+}. There was a gradual increase in inorganic phosphate liberation with incubation time. Similarly, phytase of *S. thermophile* was able to hydrolyze various insoluble metal-phytates efficiently and the hydrolysis rate was higher at 60 °C as compared to 26 °C [18]. A similar, but lower rate of hydrolysis of insoluble phytate salts by fungal, bacterial, and wheat phytases was observed by Tang et al. [20].

Phytic acid is a well known anti-nutritional factor forming insoluble complexes with metal cations, carbohydrates and proteins. These insoluble complexes are not digested in monogastric animals and, therefore, less likely to interact with phytase [22]. For this reason, phytin is often considered to be an anti-nutrient because of its ability to bind with other nutrients rendering those nutrients as well as the phosphorus contained in the phytin molecule partially or completely unavailable to the monogastric animals. Most proteins of plant origin, such as those derived from soybean, peanut and cottonseeds form complexes with these proteins [22]. Thus, the protein–phytate complex showed turbidity in a wide range of pH from 5.0–8.5. Protein–phytate complex (phytic acid and lysozyme) appeared as a turbid solution due to the formation of

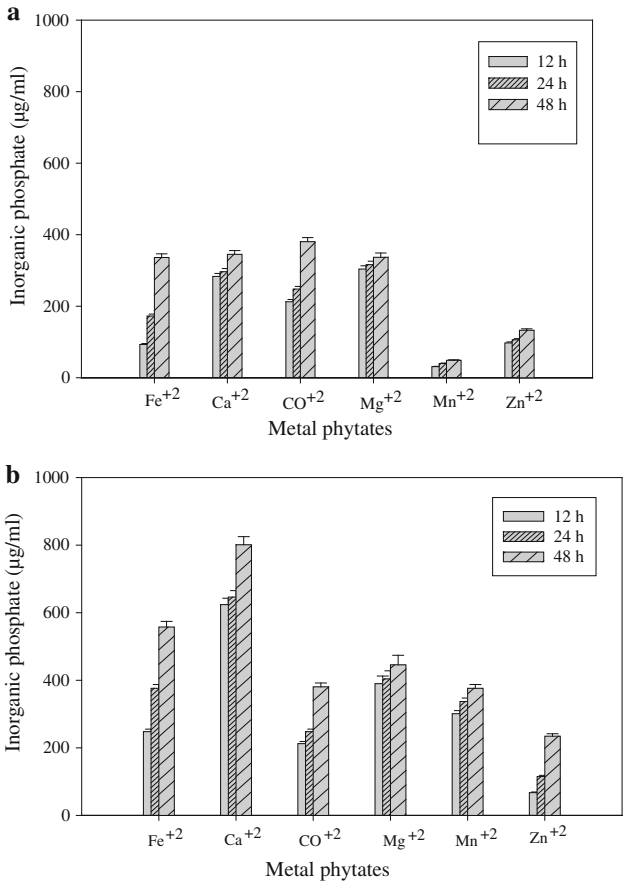

Fig. 8 **a** Hydrolysis of insoluble metal-phytates by the phytase of *A. oryzae* at 25 °C, **b** hydrolysis of insoluble metal-phytates by the phytase of *A. oryzae* at 50 °C

Table 3 Effect of proteases (1 %) on phytase activity

Treatment	Incubation time and phytase activity (U/ml)			
	0 min	30 min	60 min	120 min
Control	2.52	2.43	2.55	2.29
Pepsin	3.28	3.10	2.91	3.20
Trypsin	2.22	1.77	1.62	1.38

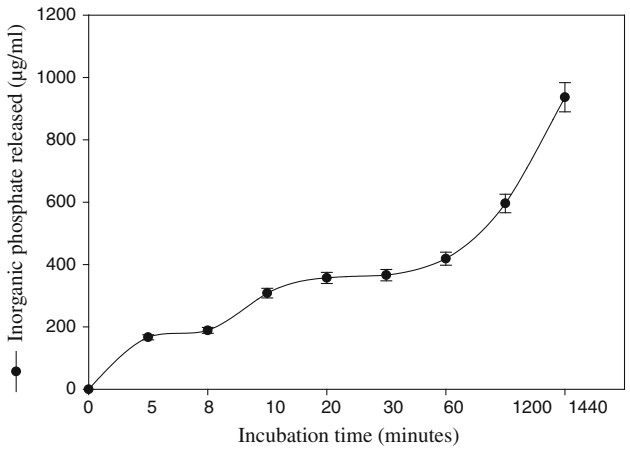

Fig. 9 Profile of liberation of inorganic phosphate from protein–phytate complex by the action of phytase of *A. oryzae* at 30 °C

insoluble complexes [22]. The absorbance of this complex at 600 nm showed a time dependent decrease from 1.65 to 0.28 within 15 min after treatment with phytase of *A. oryzae* at 30 °C. This decrease in absorbance was obtained due to the hydrolysis of protein–phytate complex by the action of phytase. This hydrolysis pattern was further supported by liberation of inorganic phosphate. The amount of inorganic phosphate linearly increased with increase in reaction time. A similar observation was made by Tran et al. [22] for the hydrolysis of protein–phytate complex of phytate with lysozyme, BSA and trypsin. Furthermore, the hydrolysis of protein–phytate complexes was observed under Scanning Electron Microscopy. There was a time dependent hydrolysis of phytate complexes by the action of *A. oryzae* phytase (Fig. 9). Moreover, this is the first study revealing the hydrolysis of insoluble phytate

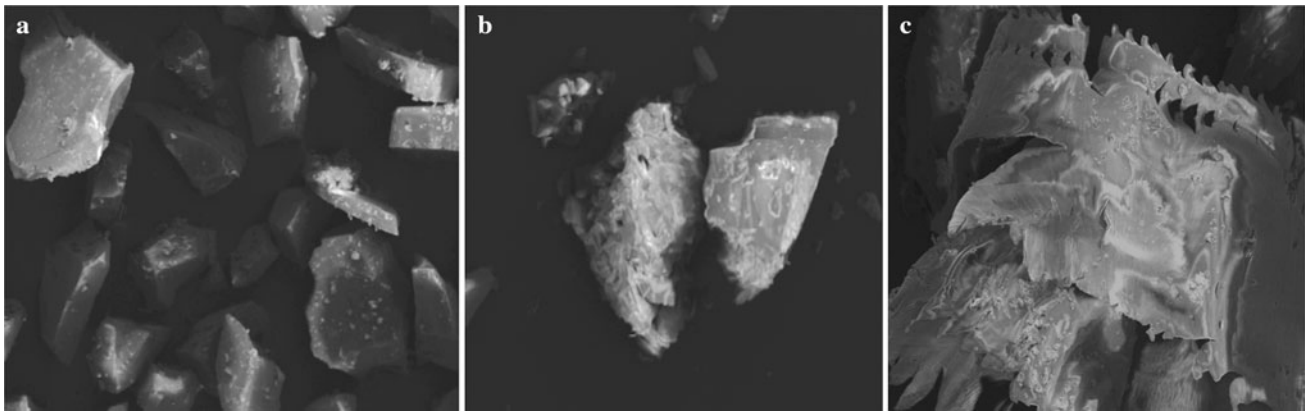

Plate 1 Scanning electron micrograph showing the hydrolysis of insoluble metal–phytate complexes. **a** Untreated sample at a magnification of ×250, **b** phytase treated samples after 60 min at a magnification of ×350 showing the cracking as a result of hydrolysis of insoluble metal-phytate, **c** micrograph showing hydrolysis of the metal–phytate complex after 120 min at a magnification of ×350

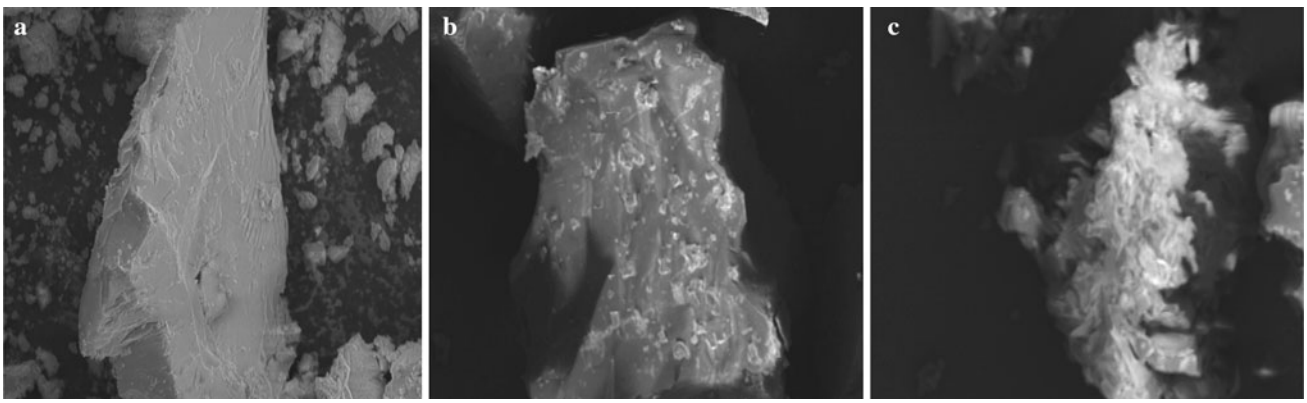

Plate 2 Scanning electron micrograph showing the hydrolysis of insoluble protein–phytate complexes. **a** Untreated sample at a magnification of ×500, **b** Phytase treated samples showing the beginning of hydrolysis of insoluble phytate complex with lysozyme after 60 min at a magnification of ×500, **c** Micrograph showing more than 90 % hydrolysis of the phytate–lysozyme complex after 120 min at a magnification of ×500

complexes with the help of scanning electron microscopy (Plate 1, 2).

Conclusions

Among 300 isolates of filamentous fungi, *A. oryzae* SBS50 produced higher phytase as compared to other fungi. Optimization of cultural parameters resulted in a 9-fold improvement in phytase production. Addition of Tween 80 resulted in a further 5.4-fold enhancement in phytase production. *A. oryzae* is a GRAS microorganism which could find application for improving nutritional quality of food/feed with concomitant reduction in an anti-nutritional factor, i.e., phytic acid. Phytase being thermostable, acid stable and protease resistant could be an ideal additive for food and feed for monogastrics. Furthermore, the enzyme efficiently hydrolyzed insoluble phytates, which are present in plant derived food ingredients as well as in soil. Therefore, the enzyme in addition to feed additive could be used in soil amendment for promoting the growth of plants making phosphorus available from insoluble phytates.

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