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# OPTIMIZATION OF CULTURE CONDITIONS FOR THE PRODUCTION OF PHYTASE FROM *Aspergillus heteromorphus* MTCC 10685

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## ABSTRACT

*Aspergillus heteromorphus* MTCC 10685, a novel phytase producer was isolated from dumps of poultry farms collected from Meerut (UP). In the present study, phytase production from *Aspergillus heteromorphus* and optimization of the culture conditions to enhance the phytase production has been reported. Among the eight fungal phytase producers isolated from various sources, the fungal strain 'K' identified as *Aspergillus heteromorphus* exhibited highest phytase activity of 14.20 U/ml. To enhance the production level of the enzyme, different culture conditions were optimized and observed that optimum temperature and pH for phytase production was 30$^{o}$C and 6.0 respectively. Maximum growth as well as enzyme production was recorded after 120 hrs of incubation period in the medium containing 0.1% calcium phytate. Yeast extract and casein (0.3%) among the organic nitrogen sources while ammonium sulphate (0.3%) as inorganic nitrogen source were found to be the best nitrogen source. Among the additives, metal ions $Ca^{++}$, $Na^{+}$, $K^{+}$, $Mg^{++}$ and $Mn^{++}$, EDTA and toluene did not affect enzyme production. However, metal ions like $Co^{++}$, $Zn^{++}$, $Ni^{++}$, $Ba^{++}$, $Pb^{+++}$, $Hg^{++}$, $Ag^{++}$ and the various detergents tested, inhibited the production of phytase. The optimization of culture conditions enhanced the production level of phytase (24.88 U/ml) by 1.75-fold.

**Key words:** *Aspergillus heteromorphus*, calcium phytate, phytase production, submerged fermentation, yeast extract

## [I] INTRODUCTION

Phytic acid (*myo*-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate) and mixed cations salts of phytic acid, designated as phytates, are a group of organic form of phosphorus, which comprises 1 to 5% by weight of edible legumes, cereal grains, oilseed meals, pollens and nuts [21,31]. Most foods of plant origin contain 50 to 80% of their total phosphorus as phytate [7]. It acts as an anti-nutritional factor, since it causes mineral deficiency due to efficient chelation of metal ions such as $Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$ and $Fe^{2+}$. It also forms complexes with proteins, affecting their digestion and also inhibits enzymes like amylase, trypsin, acid phosphatase and tyrosinase [5,7,13]. Due to the lack of adequate levels of phytases in the gut of monogastric animals (poultry, pigs, fishes and

humans), phytic acid is excreted in faeces, which is degraded by soil micro-organisms releasing phytate phosphorus in the soil that reaches aquatic bodies leading to eutrophication and algal blooms [4, 27]. Therefore, supplemental inorganic phosphate is added to their feed to fulfill the phosphate requirement and to ensure good growth. However, supplemental inorganic phosphate does not diminish the antinutritive effect of phytic acid. The ruminants digest phytic acid through the action of phytases produced by the anaerobic gut fungi and bacteria present in their rumen microflora. Monogastric animals such as pig, poultry and fish utilize phytate phosphorus poorly because they are deficient of phytases in gastrointestinal tract.

These problems could be solved by hydrolysis of phytate using supplemental phytase [19]. Therefore, phytase has become an important industrial enzyme and is the object of extensive research. By the addition of supplemental phytase in feed the antinutritive effects of phytic acid could be diminished and the cost of diets also reduces by removing or reducing the need for supplemental inorganic phosphate. In addition, phytase would be an environmentally friendly product, reducing the amount of phosphorus entering the environment thereby reduces the phosphorus pollution created by monogastric livestock production [33].

Phytases (*myo*-inositol hexakisphosphate 3-phosphorylase, EC 3.1.3.8 and *myo*-inositol hexakisphosphate 6-phosphorylase, EC 3.1.3.26) are classified as the family of histidine acid phosphatases that catalyze the hydrolysis of phytic acid to inorganic phosphate and myo-inositol phosphate derivatives in a stepwise manner [14,31]. The reduction of phytic acid content in the food and feeds by enzymatic hydrolysis using phytase is desirable. This enzyme has, therefore, potential applications in feed and food industries. Phytase enzymes are widespread in nature, occurring in microorganisms, plants, as well as in some animal tissues [9,17,20,21,31]. Phytase has been isolated from number of micro-organisms like bacteria, moulds and yeasts. The fungal isolates belonging to the genera *Aspergillus, Penicillium, Mucor* and *Rhizopus* are the most active microorganisms capable of producing phytase through submerged and solid-state fermentation [12]. But there is a constant search for phytase with more desirable properties for commercial applications. This paper describes the studies on optimization of culture conditions for phytase production by *Aspergillus heteromorphus* MTCC 10685 in submerged fermentation. This is the first report on the production of phytase from *Aspergillus heteromorphus*.

## [II] MATERIALS AND METHODS

### 2.1 Chemicals

Calcium phytate (calcium salt of phytic acid) was purchased from HiMedia Chemical Laboratories Pvt. Ltd. Company, Mumbai. All other chemicals used were of analytical grade and obtained from leading manufacturers including BDH, Sigma and Glaxo.

**2.2 Micro-organism:** The phytase producing fungal strain used in the present investigation was isolated from soil dumps of poultry farm, Meerut (UP). The fungal strain 'K' was identified as *Aspergillus heteromorphus* (MTCC no. 10685) from Microbial Type Culture Collection, IMTECH, Chandigarh (India). The fungus was maintained at $4^{o}$C on Saboraud's agar (SDA) medium which consists of peptone (1%), dextrose (4%) and agar (1.5%).

**2.3 Isolation of phytase producing fungi:** The seven different soil samples were collected from dumps of poultry places, farms and gardens. For isolation of phytase producing fungi, one gram of soil sample was suspended in 100 ml of sterile distilled water. The suspension was shaken well at 200 rpm for 5 min and 1 ml of the suspension was transferred to 9 ml sterile water blank. This procedure was repeated to obtain a dilution of 1/100,000 times ($10^{-5}$). 0.1ml of the appropriate

dilutions were spread on the Saboraud's dextrose agar (SDA) plates and incubated at 30°C for 5-7 days. Different fungal colonies appeared on the plates after incubation. All morphologically contrasting colonies were purified. These fungal isolates were screened for their phytase producing capability by qualitative and quantitative method.

**2.4 Screening of phytase producing fungi**

The qualitative screening was carried out on PS medium (phytase screening medium containing (g/L) Glucose-10.0; $(NH_4)_2SO_4$-3.0; KCl-0.5; $MgSO_4.7H_2O$-0.5; $CaCl_2$-0.1, Calcium phytate, 5.0; pH 5.5) by observing the zone of phytate hydrolysis [9]. The isolates producing zone around its growth on PSM agar plates (PS medium supplemented with 0.5% calcium phytate) were selected as phytase producers. The slants with luxuriant growth were stored at 4°C.

Acid producing fungi also solubilize calcium phytate, so, a counterstaining technique was used to identify the phytate-hydrolysing ability of the microbes [2]. Briefly, the technique involved flooding of the plate with 2% cobalt chloride for 5 min, after which the solution was replaced and incubated for 5 min at room temperature with freshly prepared colouring reagent containing equal volumes of 6.25% (w/v) aqueous ammonium molybdate solution and 0.42% (w/v) ammonium vanadate solution. Cultures exhibiting zone of clearance after removal of the chromogen were confirmed as phytase producers.

**2.5 Inoculum preparation for fermentation**

Spore inoculum was prepared from fully sporulated (7 days) old SDA slant culture. To fully sporulated agar slope culture, 20 ml of sterile distilled water with 0.01% (v/v) Tween-80 was added. The spores were scrapped using an inoculation needle under aseptic conditions. The spore count in the suspension was $10^7$spores/ml. 1ml of the spore suspension (2%) was used as inoculum.

**2.6 Phytase production:**

The fungal isolates exhibiting largest zone of clearance on the agar plates were selected and further subjected to quantitative screening for extracellular phytase production in phytase screening (PS) broth supplemented with 0.1% calcium phytate as sole source of phosphorus. Erlenmeyer flasks (250ml) containing 50ml PS broth were inoculated with 1.0 ml of spore suspension and incubated at 30°C for 5 days at 200rpm. After incubation, the culture was filtered through Whatman no. 1 filter paper. The phytase activity from the culture supernatant was determined by performing phytase assay. The fungal isolate showing highest phytase activity was selected for further studies.

**2.7 Phytase assay:**

Phytase activity was estimated by colorimetric method. Phytase was assayed using calcium phytate as substrate. The calcium phytate (0.2%) prepared in 0.1M acetate buffer (pH 5.0) was used as a substrate. The reaction mixture was prepared by the addition of 600 µl substrate with 150 µl of the crude enzyme and incubated at 35ºC for 20 minutes. The enzymatic reaction was stopped by adding 750 µl of 5% (w/v) trichloroacetic acid solution and the released free orthophosphate (Pi) in the reaction mixture was measured by a modification of the method of Fiske and Subbarow [6]. Colour reagent (750 µl), prepared daily by mixing four volumes of 1.5% (w/v) ammonium molybdate in a 5.5% (v/v) sulfuric acid solution and one volume of a 2.7% (w/v) ferrous sulphate solution, was added to the sample solution (750 µl) and the production of phosphomolybdate was measured spectrophotometrically at 700 nm [2].

One unit of phytase is defined as the amount of enzyme that liberates one µg inorganic phosphate/ml/min under the assay conditions.

**2.8 Biomass determination:** To determine the fungal biomass, the culture was filtered through Whatman No. 1 filter paper. The filtered mycelium was dried at 60°C for 24 hr in a pre-weighed dry petridish to constant weight and expressed as g dry weight/L medium.

**2.9 Optimization of culture conditions for phytase production:** The following culture conditions were optimized to enhance phytase production from *Aspergillus heteromorphus* MTCC 10685.

**Screening of different medium for enzyme production:** To screen the medium for maximum production of phytase, different production medium viz. medium A, B, C, D and E were tested. The composition of different medium was as follows (g/L):

**Medium A**: Phytase screening (PS) broth supplemented with 0.1% calcium phytate (pH 5.6) [30].

**Medium B** (Phytase screening medium, PSM): Glucose, 15.0; $NH_4NO_3$, 5.0; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $FeSO_4.7H_2O$, 0.01, $MnSO_4.7H_2O$, 0.01, calcium phytate, 5.0 (pH 5.5) [9].

**Medium C** (Starch medium): Corn starch, 80.0; Glucose, 30.0; $NaNO_3$, 8.6; $KH_2PO_4$, 0.017; KCl 0.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.1 (pH 5.0) [32].

**Medium D**: Glucose phosphate (GP) broth: Glucose, 10.0; $(NH_4)_2SO_4$, 3.0; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $CaCl_2$, 0.1; $KH_2PO_4$, 3.0; trace elements (pH 5.6) [30].

**Medium E**: Peptone dextrose (PD) broth: Peptone, 10.0; dextrose, 40.0; (pH 5.6) [30].

**Effect of incubation period:** To study the effect of incubation period, the fungal culture *Aspergillus heteromorphus* was inoculated in medium A and incubated at 30ºC for 7 days (200 rpm) under submerged condition. The samples were harvested at regular time intervals of 24 hrs and cell growth (biomass) as well as enzyme activity was measured.

**Effect of medium pH and temperature on enzyme production:** The optimum pH of the culture medium and incubation temperature for phytase production was determined in the pH range of (3.0-10.0) and ($25^o$-$50^o$C) respectively. After incubation in these conditions, phytase activity was determined from the culture filtrate.

**Effect of substrate concentration:**

The production of extracellular fungal phytase was induced by limiting concentration of inorganic phosphate in the growth medium. Calcium phytate was used as substrate or inducer for phytase production by *Aspergillus heteromorphus.* To determine the optimum substrate concentration for maximum enzyme production different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6%) of calcium phytate were tested and growth as well as phytase activity was determined.

**Effect of inoculum size:**

In order to investigate the effect of different inoculum size on phytase production by *Aspergillus heteromorphus*, Erlenmeyer flasks (250 ml) containing 50 ml medium A were inoculated with different inoculum percentage (1, 2, 3, 4 and 5%, v/v) and incubated at $30^o$C for 5 days. The growth and phytase activity were determined as mentioned earlier.

**Effect of carbon source:**

To study the effect of different carbon sources on phytase production, the medium was supplemented with different sugars (at 1% w/v concentration) such as glucose, sucrose, maltose, lactose, galactose, cellulose, cellobiose, soluble starch, myo-inositol, glycerol and fructose. The production medium with 0.1% calcium phytate and glucose was kept as control. These flasks were incubated at $30^o$C for 120 hrs and phytase activity and biomass production were estimated.

**Effect of nitrogen source:**

Effect of nitrogen supplements (0.3% w/v) like inorganic nitrogen sources such as ammonium sulphate (control), ammonium nitrate, ammonium chloride, ammonium acetate, ammonium oxalate, ammonium ferrous sulphate, sodium nitrate and organic nitrogen sources such as casein, urea, peptone, tryptone, yeast extract, beef extract, malt extract and soybean meal on phytase production from *Aspergillus heteromorphus* was studied.

The production medium A was supplemented with different inorganic and organic nitrogen sources and inoculated with freshly prepared 2%

inoculum of *Aspergillus heteromorphus.* Phytase activity was determined after 5 days.

**Effect of metal ions:** The effect of metal ions (0.1% w/v) on enzyme production was studied by adding different salts viz. NaCl, KCl, $CaCl_2$, $MgCl_2$, $MnCl_2$, $ZnSO_4$, $NiCl_2$, $CoCl_2$, $Pb(NO_3)_2$, $BaCl_2$, $HgCl_2$ and $AgCl_2$ in the production medium. After incubation of the culture in these conditions, phytase activity was determined as described earlier.

**Effect of additives (surfactants and solvents):** The effect of additives (detergents and solvents) (0.1% w/v) on enzyme production was studied by adding detergents viz. Tween-20, Tween-40, Tween-60, Tween-80, Triton X-100, sodium dodecyl sulphate (SDS), ethylenediaminetetraacetic acid (EDTA) and solvents like β-mercaptoethanol, dithiothreitol (DTT), toluene and glycerol, in the production medium and phytase activity was determined from the culture filtrate.

**2.10 Result analysis:** All the fermentations and assays were carried out in triplicate and the mean values are presented.

## [III] RESULTS AND DISCUSSION

### 3.1 Isolation and screening of phytase producing fungal strains

Thirty nine fungal strains were isolated from the seven soil samples on SDA plates. On the basis of the clear zone formation around the colonies on PSM agar plates (PSM supplemented with 0.5% calcium phytate), eight fungal isolates were selected as phytase producers. Further extracellular phytase production from these strains was studied in liquid medium. The soil isolate designated as culture K produced highest phytase activity (14.80 U/ml) and hence selected for further studies (Fig.1). It was identified as *Aspergillus heteromorphus* (MTCC no. 10685) by MTCC, IMTECH, Chandigarh (India).

### 3.2 Optimization of culture conditions for maximum phytase production

The proper combination of various cultural conditions was established in order to achieve maximum phytase production by *Aspergillus heteromorphus*. The different parameters optimized were as follows:

**Screening of different medium for phytase production**

Five production media viz. medium A, B, C, D and E were tested in an attempt to improve the phytase production from *Aspergillus heteromorphus***.** Among the different medium tested, growth as well as enzyme production (14.80 U/ml) was found to be maximum in medium A (PS broth supplemented with 0.1% calcium phytate) used initially and was comparable in medium B (Table 1). The enzyme production was supported to an appreciable level in medium D and was found minimum in medium C. No growth however was observed in medium E. The results suggest that the phytase from *Aspergillus heteromorphus* is inducible in nature, since all the media tested contained the source of phosphorus except medium E (PD medium). In contrast to these results, Vohra and Satyanarayana recorded highest phytase production (14U/g dry biomass) from *Pichia anomala* in peptone dextrose (PD) broth as compared to the various media supplemented with phosphate source [30].

Howson and Davis tested extracellular phytase activity in the fungal strains of genera *Aspergillus, Rhizopus, Mucor, Geotrichum* and *Saccharomyces* in phytase screening (PS) broth and potato dextrose medium and detected 12 strains out of 84 produced phytase in both medium indicating that the enzyme was constitutive. Twenty one strains showed phytase activity only in PS broth indicating that the enzyme was induced by the presence of substrate, while 25 strains showed activity only in potato dextrose medium filtrates [9].

**Time course of phytase production**

The time course of phytase production was studied to determine the optimum incubation time required for maximum phytase production. The phytase activity was initially detected at 48 hr and

progressively increased with time. Maximum growth as well as phytase production (17.88 U/ml) was observed at 120 hrs of incubation (Fig.2). Thereafter, the enzyme production slightly decreased. The enzyme yield on prolonged incubation decreased which could be due to reduced nutrient level of medium or autolysis of the mycelium occurred. Shimizu also observed maximum phytase activity (0.4 U/ml) at 4-5 days of fermentation by *Aspergillus oryzae* K1 [18]. Singh and Satyanarayana also found maximum phytase production at 5 days of incubation by thermophilic mould *Sporotrichum thermophile* BJTLR50 [22]. However, Howson and Davis reported highest extracellular phytase activity from *Aspergillus ficuum* NRRL 3135 after 10 days of incubation [9]. Similarly, maximum phytase activity of 68 U/ml was reported from *Aspergillus niger* NCIM 563 on the 11th day of fermentation [3].

**Effect of incubation temperature**

The effect of incubation temperature on phytase production from *Aspergillus heteromorphus* was studied in the temperature range of 25º-50ºC under submerged fermentation conditions. The optimum temperature for growth and phytase production from *Aspergillus heteromorphus* was found to be 30ºC (Table 2). Further rise in temperature, decreased the production of phytase and the minimum phytase activity was observed at 40ºC. Above 40ºC, the culture could not grow. The fermentation temperature for optimum production of phytase is mostly reported to be 30°C [1, 23,25,29,32].The optimum temperature of the yeast *Candida krusei* for growth and phytase production was also found to be 30°C [15].

Howson and Davis reported phytase production from *Aspergillus niger* at 25°C [9]. Sano *et al.* found that *Arxula adeninivorans* secreted phytase optimally at 28°C [16]. Some reports also mentioned phytase production at temperature range of 35° to 50°C. Hassouni *et al.* reported highest phytase activity at 45°-50°C by thermophilic filamentous fungi *Myceliophthora thermophila* [8].

**Effect of pH of the culture medium**

The optimum pH for phytase production from *Aspergillus heteromorphus* was found to be 6.0 and further decrease in pH of the medium reduced the enzyme production drastically (Table 3). The fungus however, could grow in pH range of 5.0-10.0 exhibiting sufficient phytase activity in the alkaline range (8.0-10.0). Though, phytase production at alkaline conditions has not been reported so far. Kim *et al.* and Tahir *et al.* also obtained maximum phytase activity from *Aspergillus* sp. 5990 and *Aspergillus niger* St-6 respectively in the production medium of pH 6.0 [11,25]. Phytase production was mostly reported that acidic to neutral pH range. *Aspergillus niger* strain 89 produced phytase optimally at pH 3.0 [32], while *Arxula adeninivorans* at pH 5.5 [16]. *Aspergillus. oryzae* K1 and *Bacillus* sp. DS11 produced highest phytase titres at pH 6.5 [10,18].

**Effect of substrate concentration on enzyme production**

Substrates are known to induce and thus, enhance enzyme production. As evident from Table 1, the phytase from *Aspergillus heteromorphus* is inducible in nature. The optimum substrate concentration for enzyme production was determined by supplementing the medium A with the calcium phytate in the range of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6%. The maximum enzyme production (21.80 U/ml) was found at 0.1% calcium phytate, growth however was observed maximum at 0.2-0.3% (Fig 3). Tambe *et al.* obtained highest phytase production by *Klebsiella aerogenes* in the medium containing 2% sodium phytate [26]. The enzyme produced by *Sporotrichum thermophile* BJTLR50 was also found to be inducible nature, as the enzyme titres enhanced many folds at 0.3% concentration of sodium phytate in the medium [22].

**Effect of inoculum size**

The effect of different inoculum size (1, 2, 3, 4 and 5%) on phytase production was studied and

the optimum inoculum size was found to be 2% (Fig 4). The enzyme production (21.84 U/ml) enhanced as compared to 1% inoculum used initially. At higher inoculum levels, the enzyme production declined due to competition among the fungal population for the nutrients. Singh and Satyanarayana has reported maximum phytase production by using inoculum ($1x10^7$ spores/50ml medium) of thermophilic mould *Sporotrichum thermophile* BJTLR50 [22].

**Effect of carbon source**

To obtain the maximum phytase production from *Aspergillus heteromorphus* different sugars as carbon source were tested. Glucose was found to be the best carbon source (21.21 U/ml), however starch (20.12 U/ml) followed by sucrose (19.05 U/ml) produced comparable enzyme activity. Galactose as carbon source did not support growth as well as enzyme production (Table 4). Galactose was also found inhibitory for phytase by *Pichia anomala*, while glucose (4%) supported maximum enzyme production [30]. Singh and Satyanarayana also studied the effect of different carbon sources. Among various carbon sources tested, glucose supported highest phytase production from *Sporotrichum thermophile* as compared to other carbon sources [22]. Shieh and Ware (1968) and Tahir *et al.* (2010) found sucrose as the best carbon source for phytase production [17, 25]. *Aspergillus niger* produced high phytase titres, when grown in a medium containing corn starch along with glucose [32]. However, phytase production from *Arxula adeninivorans* increased to several folds using galactose as carbon source as compared to glucose [16].

Among the different concentration of glucose ranging from 0.5 to 5.0%, the optimum phytase production was observed at 0.5%. Higher concentrations of glucose favoured the growth of the fungus but enzyme production inhibited strongly (Fig.5). Singh and Satyanarayana reported similar observations for phytase produced by *Sporotrichum thermophile*. Highest phytase production was observed at 1.5% (w/v) glucose; thereafter increase in concentration upto 5% decreased enzyme production sharply [22].

**Effect of nitrogen source**

The effect of nitrogen source on phytase production from *Aspergillus heteromorphus* was studied by supplementing the production medium with various inorganic and organic nitrogen (0.3%) sources. Ammonium sulphate being a constituent of medium A was kept as control. Among the various nitrogen sources tested, yeast extract produced higher phytase titres (24.88 U/ml) as compared to control (22.2 U/ml). Casein (21.9 U/ml) and ammonium nitrate (19.9 U/ml) also produced comparable phytase activity (Table 5). Sano *et al*. reported yeast extract and peptone as the best nitrogen source for *Arxula adeninivorans* phytase [16]. Similarly, Vohra and Satyanarayana also observed enhanced enzyme production by *Pichia anomala* using organic nitrogen sources in the production medium [30]. Phytase production from *Bacillus* sp. DS11, *Aspergillus niger* St-6 and *Aspergillus niger* van Teighem was found maximum with ammonium nitrate as nitrogen source [10,25,29].

**Effect of metal ions:**

The effect of metal ions (0.1% w/v) on enzyme production was studied by adding various salts in the production medium and found that the metal ions $Ca^{++}$, $Na^{+}$, $K^{+}$, $Mg^{++}$ and $Mn^{++}$ did not affect enzyme production. However, $Zn^{++}$, $Ni^{+}$, $Co^{++}$ and $Hg^{++}$ strongly inhibited the growth as well as enzyme production, while $Ba^{++}$, $Pb^{++}$ and $Ag^{++}$ inhibited the enzyme production to about 50% (Table 6). Soni *et al*. observed that $Fe^{++}$, $Cu^{++}$ and $Hg^{++}$ strongly inhibited phytase production from *Aspergillus niger* NCIM 563 [24].

**Effect of additives:**

Among the various detergents (0.1% w/v) tested, Triton X-100 and (SDS) strongly inhibited the enzyme production, while Tween-20, Tween-40, Tween-60 and Tween-80 also showed about 50% inhibition. EDTA did not affect the enzyme production whereas β-mercaptoethanol and DTT were inhibitory for phytase production (Table 7).

The presence of EDTA stimulated, while SDS inhibited the enzyme production from *Aspergillus niger* NCIM 563 as reported by Soni *et al*. [24].

### 3.3 Enzyme production using improved culture conditions

The optimization of various nutritional and cultural conditions enhanced the level of phytase production to 1.75-fold as compared to initial conditions. The results of the time-course study before and after optimization of culture conditions are shown in Fig 6.

## CONCLUSION

The fungal strain *Aspergillus heteromorphus* MTCC 10685 isolated from local poultry farm is a novel source of phytase. Phytase production by this strain is influenced by various physical and nutritional parameters. The results revealed that optimization studies resulted into 1.75-fold increase in phytase titres. Further studies will be conducted to characterize the phytase for its application in industrial processes.

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**Table 1.** Screening of different medium for maximum phytase production from *Aspergillus heteromorphus*

| Medium | Phytase activity (U/ml) |
|---|---|
| A (PS broth) | 14.20 (4.2) |
| B (PSM broth) | 12.11 (4.0) |
| C (Starch medium) | 5.12 (1.9) |
| D (GP broth) | 9.73 (3.4) |
| E (PD broth) | N.D. (1.1) |

Values in parenthesis represent Biomass (g dry wt/L)

**Table 2.** Effect of incubation temperature

| Temperature (ºC) | Phytase activity (U/ml) |
|---|---|
| 25º | 12.11 (5.34) |
| 30º | 17.82 (4.96) |
| 37º | 15.64 (4.05) |
| 40º | 0.23 (0.12) |
| 45º | ND (0.05) |
| 50º | ND (--) |

ND = Not Detected
Values in parenthesis represent Biomass (g dry wt/L)

**Table 3.** Effect of medium pH on phytase production

| pH | Phytase activity (U/ml) |
|---|---|
| 3.0 | 07.42 (3.1) |
| 4.0 | 09.92 (3.5) |
| 5.0 | 13.75 (3.9) |
| 6.0 | 17.42 (4.8) |
| 7.0 | 10.15 (5.0) |
| 8.0 | 10.80 (4.8) |
| 9.0 | 13.08 (5.2) |
| 10.0 | 12.11 (5.1) |

Values in parenthesis represent Biomass (g dry wt/L)

**Table 4**: Effect of different carbon sources

| Carbon source | Phytase activity (U/ml) |
|---|---|
| Glucose | 21.21 (2.99) |
| Sucrose | 19.05 (2.85) |
| Maltose | 14.92 (2.77) |
| Lactose | 15.75 (2.18) |
| Galactose | N.D. (0.11) |
| Cellulose | 15.75 (3.02) |
| Cellobiose | 14.92 (2.91) |
| Starch | 20.12 (2.71) |
| *myo*-inositol | 14.08 (2.86) |
| Fructose | 12.34 (2.60) |
| Glycerol | 14.92 (4.04) |

Values in parenthesis represent Biomass (g dry wt/L)

**Table 5:** Effect of different nitrogen sources

| Nitrogen sources (0.3%) | Phytase activity (U/ml) |
|---|---|
| Ammonium sulphate (control) | 22.29 (3.16) |
| Ammonium nitrate | 19.92 (2.85) |
| Ammonium chloride | 15.25 (3.51) |
| Ammonium acetate | 04.88 (3.05) |
| Ammonium oxalate | 08.25 (3.43) |

| | |
|---|---|
| Ammonium ferrous sulphate | 00.75 (1.24) |
| Sodium nitrate | 08.82 (3.45) |
| Peptone | 17.51 (3.76) |
| Tryptone | 14.12 (3.98) |
| Yeast extract | 24.88 (4.32) |
| Beef extract | 19.10 (3.86) |
| Malt extract | 17.41 (2.36) |
| Soyabean meal | 14.90 (6.22) |
| Casein | 21.98 (5.14) |
| Urea | ND (--) |

Values in parenthesis represent Biomass (g dry wt/L)

**Table 6:** Effect of different metal ions on enzyme production

| Metal ions (0.1%) | Phytase activity (U/ml) |
|---|---|
| Control | 23.66 (4.41) |
| $Na^{+}$ | 21.66 (5.26) |
| $K^{+}$ | 20.88 (5.23) |
| $Ca^{++}$ | 20.10 (7.22) |
| $Mg^{++}$ | 18.55 (4.66) |
| $Mn^{++}$ | 20.66 (4.13) |
| $Zn^{++}$ | 08.02 (2.79) |
| $Ni^{++}$ | 04.89 (2.40) |
| $Co^{++}$ | 03.54 (2.61) |
| $Ba^{++}$ | 15.71 (7.66) |
| $Pb^{+++}$ | 12.02 (6.99) |
| $Hg^{++}$ | 02.77 (1.67) |
| $Ag^{++}$ | 11.99 (4.45) |

Values in parenthesis represent Biomass (g dry wt/L)

**Table 7:** Effect of additives on phytase production

| Additives (0.1%) | Phytase activity (U/ml) |
|---|---|
| Control | 24.82 (3.4) |
| Tween 20 | 12.33 (6.9) |
| Tween 40 | 14.12 (7.2) |
| Tween 60 | 15.33 (8.1) |
| Tween 80 | 14.22 (6.1) |
| Triton X-100 | 8.15 (2.4) |
| SDS | 04.56 (2.3) |
| β-mercaptoethanol | 04.71 (2.3) |
| DTT | 06.22 (3.1) |
| EDTA | 19.32 (6.9) |
| Toluene | 10.88 (5.6) |
| Glycerol | 09.32 (5.4) |

Values in parenthesis represent Biomass (g dry wt/L)


**Fig.1** Zone of phytate hydrolysis by culture K *(Aspergillus heteromorphus)* on PSM plates

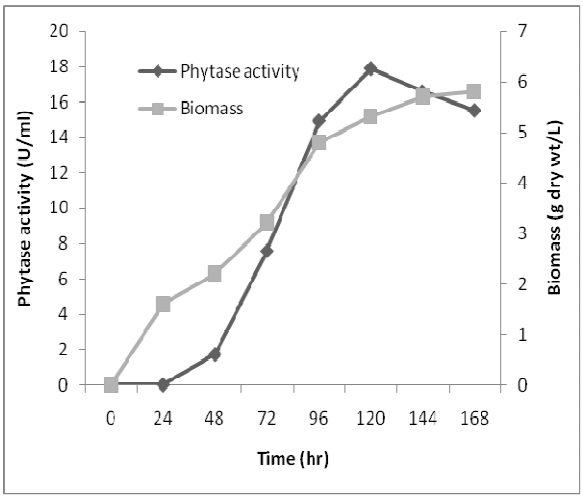


**Fig. 2** Effect of incubation period on phytase production

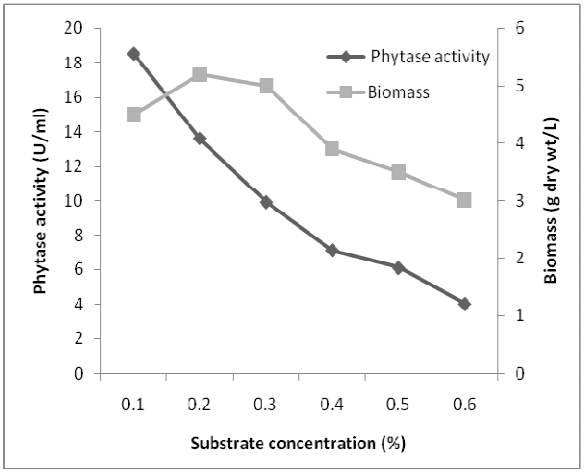


**Fig 3.** Effect of substrate (calcium phytate) concentration

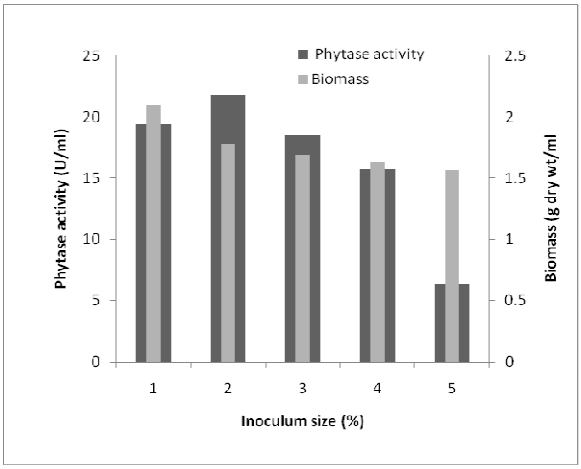


Fig 4. Effect of inoculum size

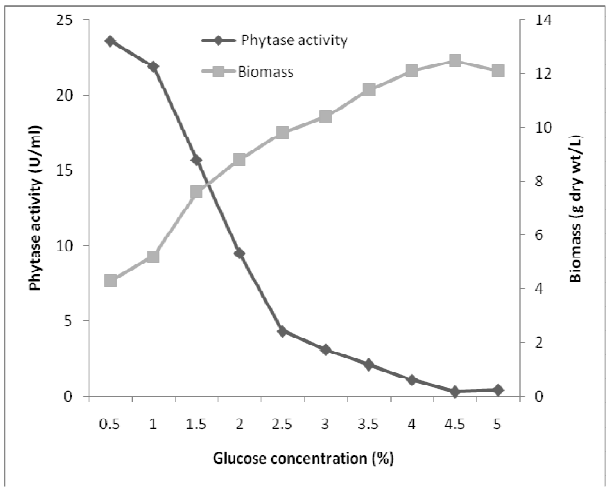


**Fig 5**. Effect of glucose concentration

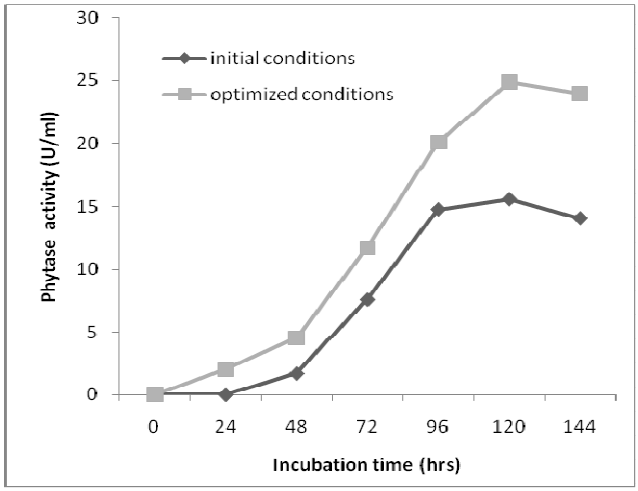


**Fig 6.** Time course of phytase production under initial and optimized culture conditions

**[**Initial conditions: PS broth with 0.1% calcium phytate, 96 hrs incubation, pH 5.5, 1% inoculum, 1% glucose as carbon source and 0.3% ammonium sulphate; Optimized conditions: PS broth with 0.1% calcium phytate, 120 hrs incubation at pH 6.0, with 2% inoculums, 0.5% glucose as carbon source and 0.3% yeast extract as nitrogen source]