Pak. J. Bot., 53(2): 763-770, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-2(12)

SCREENING OF PHYTATE DEGRADING FUNGI AND OPTIMIZATION OF CULTURE CONDITIONS FOR PHYTASE SYNTHESIS USING AGRO-INDUSTRIAL BY-PRODUCTS

SHAHZAD MAHMOOD[1,2]*, MEMUNA GHAFOOR SHAHID[1], MUHAMMAD NADEEM[2] AND IKRAM-UL-HAQ[3]

[1]*Department of Botany, Government College University, Lahore, Pakistan*
[2]*Food and Biotechnology Research Centre (FBRC), PCSIR Laboratories Complex, Lahore, Pakistan*
[3]*Institute of Industrial Biotechnology (IIB), Government College University, Lahore, Pakistan*
[*]*Corresponding author's email: shahzadbiology@gmail.com*

Abstract

The present study is concerned with the screening of microbial strains and suitable fermentation medium for the best production of phytase using solid state fermentation process. The results revealed that fermentation medium M5 gave maximum yield with *Aspergillus niger*. Different culture conditions such as effect of different carbon and nitrogen sources, different surfactants, their concentration levels, incubation period, incubation temperature, pH of the medium, moisture level and inoculum size on the production of phytase were also studied to improve the yield. The results showed that glucose (1% w/w), NH_4NO_3 (0.5% w/w) and tween-40 (0.5% w/w) were best carbon source, nitrogen source and surfactant for phytase production, respectively. Maximum activity of enzyme (297.25±7.94 IU/g) was recorded after 5 days of incubation at 35°C, pH 6, with 80% (v/w) moisture level and 10% (v/w) inoculum size, where as the enzyme activity was 198.78±5.80 IU/g under unoptimized cultural conditions. All these results indicate that optimization of culture conditions is very important to improve the production of phytase by *Aspergillus niger* using solid state fermentation process.

Key words: Phytase, *Aspergillus niger*, Culture conditions, Optimization, Solid state fermentation.

Introduction

The major portion of animal feed cereal bran, corn meal, oil seed cakes and soybean meal etc. contain 0.83-9.15% of phosphorus as the phytate phosphorus. Phytic acid is considered anti-nutritional due to its ability to chelate with divalent cations such as calcium, magnesium, iron and zinc (Bakri *et al*., 2018). Monogastric animals cannot absorb phytic acid phosphorus from plant based feed, because of insufficiency or absence of phytate degrading enzyme in their digestive system. Thus, undigested phytate phosphorus is excreted in the faecal material, which causes environmental pollution in areas of abundant livestock units. One of the alternatives for solving this problem may be supplementation of animal feed with phytase (Maguire *et al.*, 2005; Yao *et al.*, 2011).

Phytases (myo-inositol hexakis phosphate phosphohydrolase) (EC 3.1.3.8) are phosphatase enzymes that catalyze the hydrolysis of phytic acid and its salts (phytate) and generally yield *myo*-inositol pentakis-, tetrakis-, tris-, bis- and monophosphate isomers as well as liberation of inorganic phosphate and potentially chelated minerals in stepwise manner (Bohn *et al.*, 2008; Azeke *et al.*, 2011).

Phytase enzyme can be found in animals, plants, bacteria, yeast and filamentous fungi. However, among microorganisms, phytase activity has been abundantly found in filamentous fungi, particularly in *Aspergillus* species (Jafari-Tapeh *et al*., 2012; Akturk *et al*., 2013; Bhavsar *et al*., 2013; Bakri *et al*., 2018).

The use of filamentous fungi such as *Aspergillus niger* for the production of phytase through solid state fermentation (SSF) using agro-industrial wastes has gained many interests for research in the last years (Pandey *et al.*, 2001). SSF system has gained much interest because it offers several economical and practical advantages including high products concentration, improved products recovery, simple cultivation equipments and lower plant operational costs (Bakri *et al*., 2018).

Phytase enzyme has taken a very important position in biotechnological applications as it is used as an additive in the diets of non-ruminants to reduce the phytate content of fodder and commercial foods (Omogbenigun *et al*., 2003), increase bio-availability of phosphorus and other important minerals in livestock feed, preserve non-renewable phosphorus sources by reducing its need of supplementation in diets and reduce environmental pollution (Yao *et al.*, 2011).

The present study was focused on the screening of the microbial strains and the optimization of culture conditions for enhanced production of phytase under solid state fermentation process using agro-industrial by-products as substrate.

Materials and Methods

Collection of Agro-industrial by-products: In the present study, different Agro-industrial by-products i.e. wheat bran, wheat straw, rice bran, rice husk, rice polish, rice straw, cotton seed meal, soybean meal, corn cobs and corn husk were collected from local market of Lahore to find the suitable substrate for phytase production by different microbial strains.

Procurement and maintenance of microbial cultures: Different microbial strains i.e. *Penicillium commune*, *Rhizopus oligosporus, Aspergillus niger*, *Trichoderma viride* and *Saccharomyces cerevisiae* were obtained from the Microbiology Laboratory, Food and Biotechnology Research Centre (FBRC), PCSIR Laboratories complex, Lahore, and from the Department of Botany, GC University Lahore, Pakistan. The microbial strains were grown on freshly prepared Potato-Dextrose-Agar (PDA) slants for five days at 37°C in an incubator and stored at 4°C in a cool cabinet.

Screening of microbial strains and fermentation media: For the screening purpose, five microbial strains mentioned above were cultured on ten different fermentation media i.e., M1, M2, M3, M4, M5, M6, M7, M8, M9 and M10 using solid state fermentation process to find out the best producer and fermentation medium for maximum phytase production. All fermentation media after moistened with suitable amount of diluent (Distilled water) having pH 5.5 were taken in 250 ml Erlenmeyer flasks separately and mixed thoroughly (Table 1). The flasks were sterilized at 121°C for 15 minutes and cooled at room temperature. 10% (v/w) of spore suspension of each microbial strain was transferred aseptically to respective flask containing above mentioned fermentation medium and incubated at 35°C for 5-7 days.

Optimization studies of fermentation conditions for phytase production: Various fermentation conditions were screened and optimized to obtain the best production of phytase. Different parameters affecting phytase production were as follows:

Effect of different carbon sources: Different carbon sources e.g. glucose, fructose, sucrose, maltose, glycerol and starch 1% (w/w) each were used in the fermentation medium to find the suitable carbon source for maximum phytase production by *Aspergillus niger*.

Effect of different nitrogen Sources: In order to get the maximum production of phytase, different organic nitrogen sources i.e. peptone, yeast extract, malt extract, tryptone, urea and inorganic nitrogen sources i.e., $(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl, CH_3COONH_4 and $NaNO_3$ at 0.5% (w/w) concentrations were used in fermentation medium and best nitrogen source was selected.

Effect of different concentrations of glucose: Effect of various concentration levels of glucose i.e., 0.2, 0.4, 0.6, 0.8, 1 and 1.2% (w/w) were studied to find the suitable glucose concentration level for maximum production of phytase.

Effect of NH_4NO_3 concentrations: Effect of various concentration levels of NH_4NO_3 i.e., 0.25, 0.5, 0.75, 1, 1.25 and 1.5% (w/w) were studied to find the suitable NH_4NO_3 concentration level for the maximum yield of phytase.

Screening of different surfactants: Different surfactants i.e., tween-40, tween-80, triton X-100, sodium dodecyl sulphate (SDS) and sodium lauryl sulphate (SLS) were used each at 0.5% (w/w) concentration compared to control without any surfactant in the fermentation medium to identify suitable surfactant for best phytase production.

Effect of different concentration of tween-40: Different concentration levels of tween-40 i.e., 0.25, 0.5, 0.75, 1, 1.25 and 1.5% (w/w) were optimized for best phytase production.

Optimization of different process parameters: Optimization of various process parameters was conducted to evaluate the effect of an individual parameter at a time and incorporated the selected parameter as a standard before optimizing the next parameters.

Effect of incubation period: Effect of incubation period was studied by incubating the culture medium for different time intervals (i.e. 2, 3, 4, 5, 6, 7 and 8 days). The enzyme activity was measured after each interval to find the most appropriate incubation period for maximum yield of phytase.

Effect of incubation temperature: Effect of incubation temperature was studied by incubating the culture medium at different temperatures (i.e. 20, 25, 30, 35, 40, 45 and 50ºC). The enzyme activity was measured at every temperature to find the most appropriate incubation temperature.

Effect of initial pH: Optimum pH for the production of phytase was determined by adjusting the initial pH of the culture medium at different pH levels i.e., 3, 4, 5, 6, 7, 8 and 9. Distilled water of different pH was used as diluent in fermentation medium in order to maintain different pH of the medium. 1 N NaOH/HCl were used to prepare distilled water with different pH.

Table 1. Composition of the different fermentation media used for the production of phytase by various microbial strains.

Sr. No	Ingredients (%)	Fermentation media (M)									
		M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
1.	Wheat Bran	100	-	-	-	-	-	-	-	-	-
2.	Rice Bran	-	100	-	-	-	-	-	-	-	-
3.	Cotton Seed Meal	-	-	100	-	-	-	-	-	-	-
4.	Wheat Straw	-	-	-	100	-	-	-	-	-	-
5.	Rice Polish	-	-	-	-	100	-	-	-	-	-
6.	Soybean Meal	-	-	-	-	-	100	-	-	-	-
7.	Corn Cobs	-	-	-	-	-	-	100	-	-	-
8.	Corn Husk	-	-	-	-	-	-	-	100	-	-
9.	Rice Straw	-	-	-	-	-	-	-	-	100	-
10.	Rice Husk	-	-	-	-	-	-	-	-	-	100
11.	Urea	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
12.	$MgSO_4.7H_2O$	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
13.	KCl	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
14.	$FeSO_4.7H_2O$	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Effect of inoculum size: Effect of various sizes of inoculum i.e., 5, 10, 15, 20, 25 and 30% (v/w) were evaluated in fermentation medium for the maximum yield of phytase and best size of inoculm was selected.

Effect of initial moisture content: Different levels of distilled water i.e., 20, 40, 60, 80, 100 and 120% (v/w) were used as diluent in the fermentation medium to find the best level for phytase production.

Production of phytase using optimum fermentation conditions: After optimization of all the process parameters mentioned above, production of phytase was carried out by using all the optimum cultural conditions collectively in the growth medium. After sterilization, the selected growth medium M5 was inoculated with 10% (v/w) spore suspension of *Aspergillus niger* and incubated at 35°C for 5 days.

Recovery of phytase: For extraction of crude enzyme a total of 50 ml citrate buffer (0.2 M, pH 5.5) was added to each flask containing the fermented culture. The flask material was agitated in a water bath shaker at 200 rpm for 90 min. After that, the suspension was filtered and centrifuged at 10,000 rpm for 15 minutes at 4°C. The clear supernatant was then used as crude enzyme extract for the estimation of phytase. All the experiments were conducted in triplicates.

Phytase assay: Enzyme activity was determined by using modified procedure based on method of McKie & McCleary (2016). 1% (w/v) phytic acid solution and enzyme extract 0.2 ml each was taken in a test tube and incubated at 37°C for 15 min. Then, 0.4 ml of 15% tricholoroacetic acid (TCA) was added to stop the reaction. The above mixture was then incubated at 50°C for 15 min after adding colour reagent. A blank containing 0.2 ml of citrate buffer (0.2 M, pH 5.5) instead of 0.2 ml enzyme extract was run in parallel. A spectrophotometer was used to determine the absorbance of reaction mixture at 655 nm against blank.

One phytase unit is defined as the amount of the enzyme that releases 1µM of inorganic phosphorus per milimeter per minute under the standard assay conditions.

$$\text{Enzyme activity (IU/ml/min)} = \frac{\text{Absorbance of sample} \times \text{Avg. standard factor}}{\text{Incubation period}}$$

Results and Discussion

In the present study, phytase was produced from *Aspergillus niger* by solid-state fermentation using agro-industrial by-products as substrate. Different process parameters were screened and optimized for best phytase production.

Screening of microbial strains and fermentation media for phytase production: Five microbial strains i.e. *Penicillium commune*, *Rhizopus oligosporus, Aspergillus niger*, *Trichoderma viride* and *Saccharomyces cerevisiae* were cultured on ten different fermentation media i.e. M1, M2, M3, M4, M5, M6, M7, M8, M9 and M10 using solid state fermentation, for the screening purpose. *Aspergillus niger* and M5 fermentation medium were selected as the best phytase producing fungus and best fermentation medium, respectively, with 198.78±5.80 IU/g of enzyme activity (Fig. 1).

Screening and optimization of different culture conditions for phytase production

Effect of various carbon sources: Different carbon sources e.g. glucose, fructose, sucrose, maltose, glycerol and starch were used in M5 fermentation medium for phytase production. Glucose was screened as the most suitable carbon source for best phytase production (213.29±3.92 IU/g).

Effect of different concentrations of glucose: Effects of various concentrations of glucose i.e., 0.2, 0.4, 0.6, 0.8, 1 and 1.2% (w/w) were studied for maximum production of phytase. The highest yield of phytase (215.12±5.12 IU/g) was obtained at 1% glucose level (Fig. 2).

Effect of various nitrogen sources: In order to get the maximum production of phytase different organic and inorganic nitrogen sources i.e. peptone, yeast extract, malt extract, tryptone, urea, $(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl, CH_3COONH_4 and $NaNO_3$ were used in M5 fermentation medium. Fig. 3 showed that NH_4NO_3 was found as best nitrogen source for maximum phytase production (241.79±5.84 IU/g).

Effect of different concentrations of NH_4NO_3: Different concentration levels of NH_4NO_3 i.e. 0.25, 0.5, 0.75, 1, 1.25 and 1.5% (w/w) were used for the maximum yield of phytase, 0.5% NH_4NO_3 gave maximum production of phytase (243.10±5.06 IU/g).

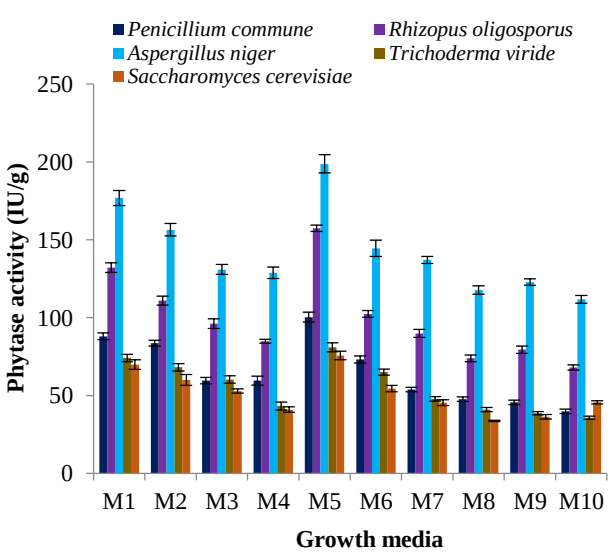

Fig. 1. Screening of microbial strains and fermentation media for the production of phytase using solid state fermentation.

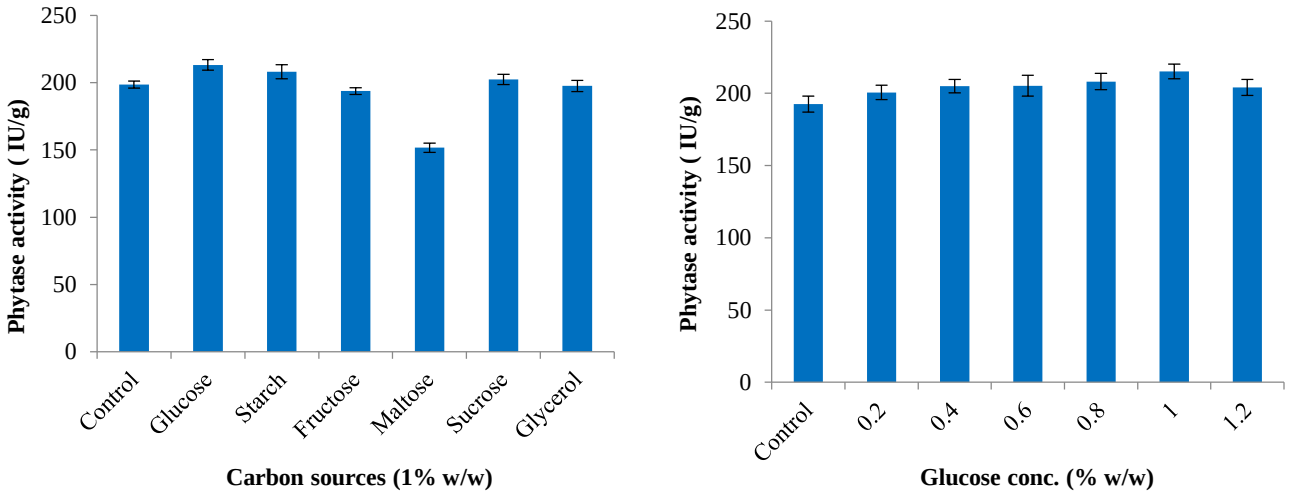

Fig. 2. Effect of carbon sources and its concentrations on phytase production by *Aspergilus niger*. Standard errors are shown. (Incubation time 5 days, Temperature 35°C, pH 5.5).

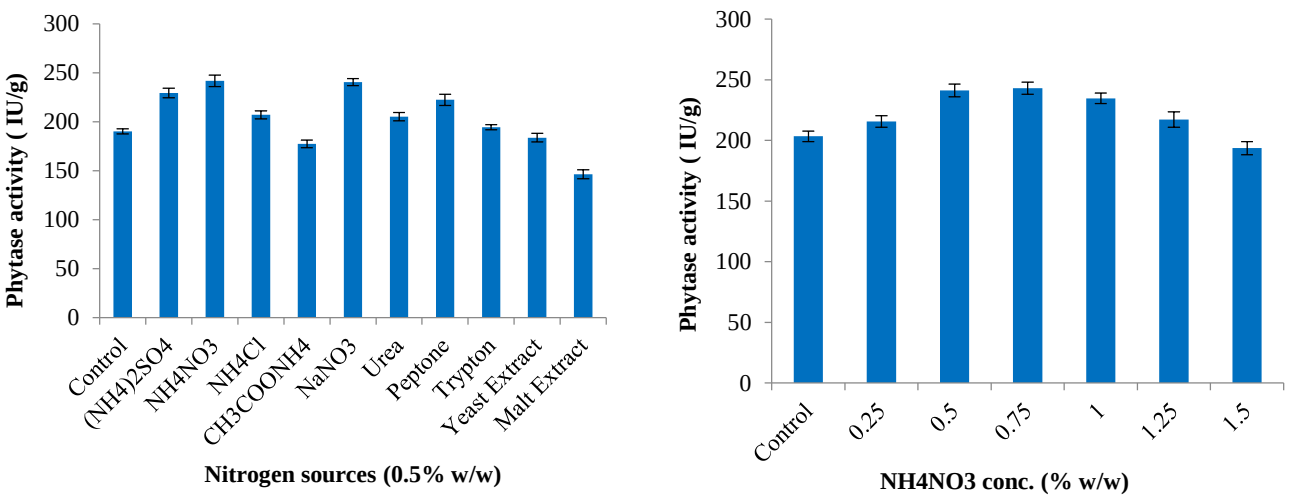

Fig. 3. Effect of nitrogen sources and its concentrations on phytase production by *Aspergilus niger*. Standard errors are shown. (Incubation time 5 days, Temperature 35°C, pH 5.5).

Effect of different surfactants: Different surfactants i.e. tween-40, tween-80, triton X-100, SDS and SLS were used in the selected fermentation medium to screen suitable surfactant for best phytase production (Fig. 4). Tween-40 showed maximum phytase production (253.04±6.16 IU/g).

Effect of different concentration of tween-40: Different concentration levels of tween-40 i.e., 0.25, 0.5, 0.75, 1, 1.25 and 1.5% (w/w) were optimized for best phytase production by *Aspergillus niger*. 0.5% tween-40 was selected as maximum phytase producer (255.08±6.15 IU/g).

Effect of incubation period: Effect of incubation period was studied by incubating the culture medium for different time intervals (2, 3, 4, 5, 6, 7 and 8 days) as shown in Fig. 5 *Aspergillus niger* gave highest production of enzyme (273.08±6.73 IU/g) after 5 days of incubation.

Effect of incubation temperature: The fermentation medium was incubated at different temperatures i.e. 20, 25, 30, 35, 40, 45 and 50ºC for best phytase production. The production of enzyme was maximum (275.49±7.36 IU/g) at 35ºC (Fig. 6).

Effect of initial pH: Initial pH of the fermentation medium was adjusted at different levels (3, 4, 5, 6, 7, 8 and 9). The phytase production was found maximum (271.85±8.41 IU/g) at pH 6 (Fig. 7).

Effect of initial moisture content: Fig. 8 showed the effect of different levels of initial moisture (i.e. 20, 40, 60, 80, 100 and 120% v/w) in the fermentation medium on phytase production and it was found that best phytase yield (276.23±4.74 IU/g) was obtained at 80% v/w of initial moisture.

Effect of inoculum size: Various inoculum sizes of *Aspergillus niger* i.e., 5, 10, 15, 20, 25 and 30% (v/w) were evaluated in fermentation medium for production of phytase. 10% (v/w) inoculum size was optimized for best enzyme production (297.25±7.94 IU/g) as represented in Fig. 9.

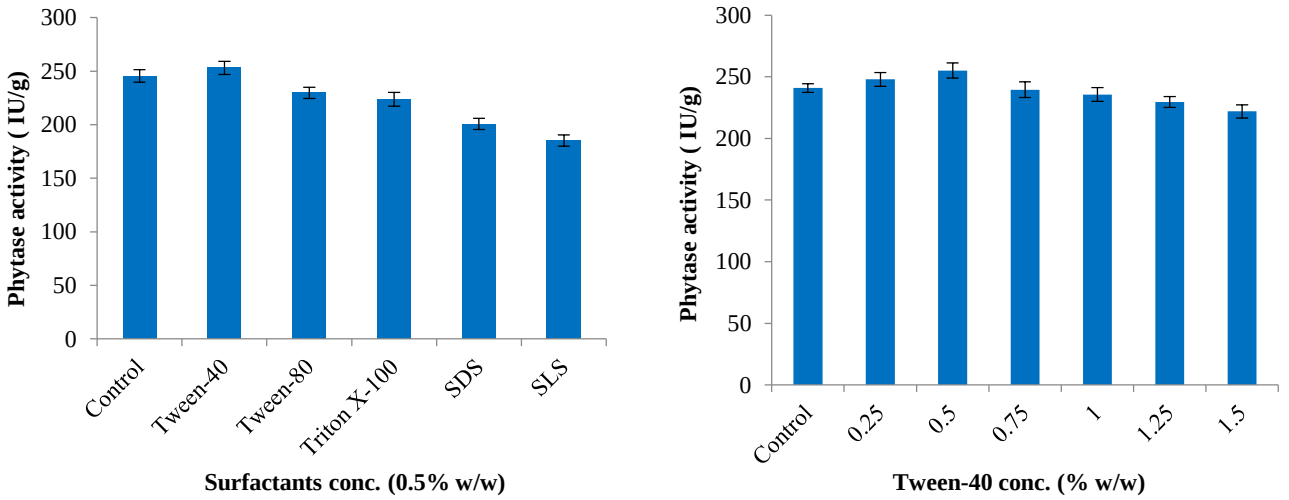

Fig. 4. Effect of different surfactants and its concentrations on phytase production by *Aspergilus niger*. Standard errors are shown. (Incubation time 5 days, Temperature 35°C, pH 5.5).

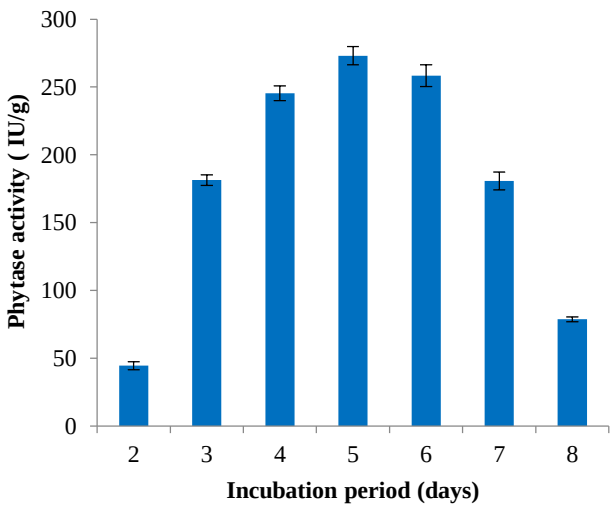

Fig. 5. Effect of incubation period on phytase production by *Aspergilus niger*. Standard errors are shown. (Temperature 35°C, pH 5.5).

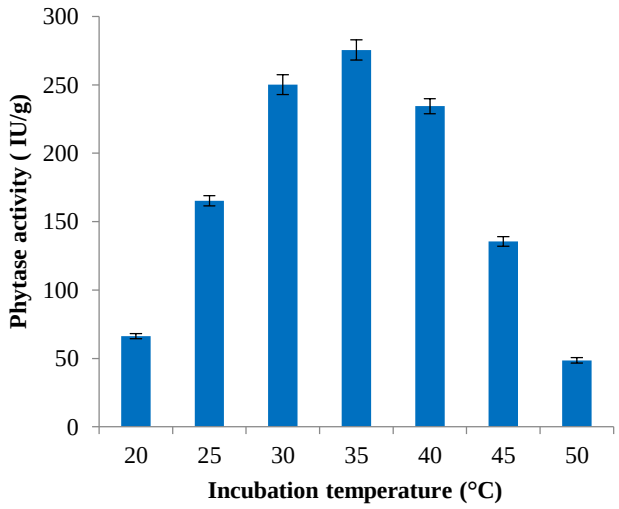

Fig. 6. Effect of incubation temperature on phytase production by *Aspergilus niger*. Standard errors are shown. (Incubation time 5 days, pH 5.5).

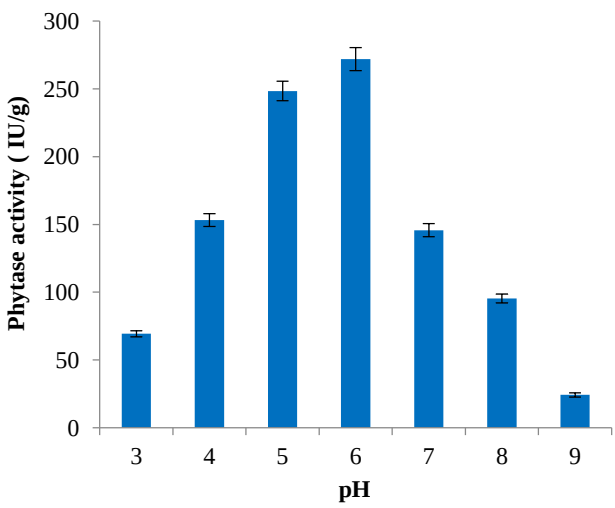

Fig. 7. Effect of Initial pH on phytase production by *Aspergilus niger*. Standard errors are shown. (Incubation time 5 days, Temperature 35°C).

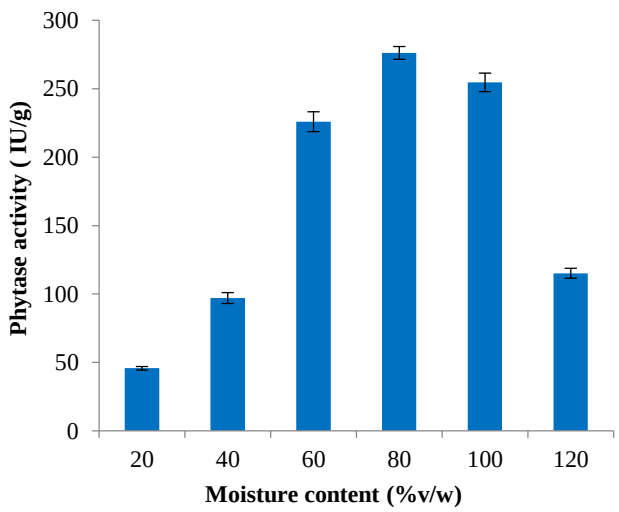

Fig. 8. Effect of moisture content on phytase production by *Aspergilus niger*. Standard errors are shown. (Incubation time 5 days, Temperature 35°C, pH 6).

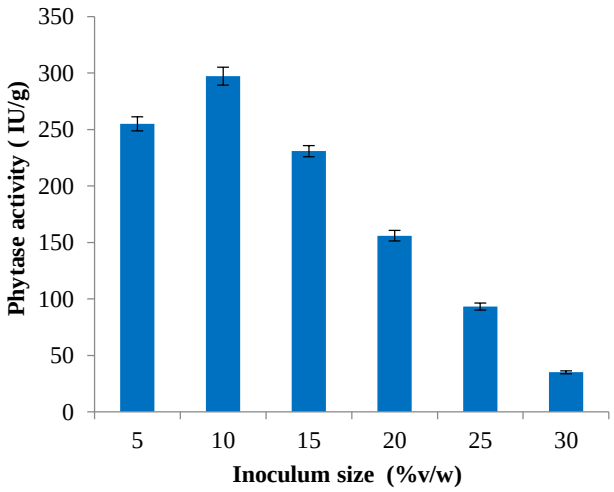

Fig. 9. Effect of inoculum size on phytase production by *Aspergilus niger*. Standard errors are shown. (Incubation time 5 days, Temperature 35°C, pH 6).

Discussion

Aspergillus niger was selected as the best microorganism amongst all the above mentioned microbial strains for the best production of phytase by solid state fermentation process. Production of phyatse was also obtained by *Aspergillus niger* CFR 335 and *Aspergillus ficuum* SGA (Gunashree & Venkateswaran, 2014) and many other species of genus *Aspergillus* reported by Singh *et al*., (2015); Buddhiwant *et al*., (2016); Thakur *et al*., (2017); Neira-Vielma *et al*., (2018); Sarita *et al*., (2018).

The microorganism must be provided with a suitable growth medium in which it can grow and produce maximum amount of enzyme (Shahid & Nadeem, 2015). In the present study, *Aspergillus niger* produced a high amount of phytase when it was grown in M5 fermentation medium (Rice polish, 1% Glucose, 0.5% NH_4NO_3, 0.1% KCl, 0.1% $MgSO_4.7H_2O$, 0.1% $FeSO_4.7H_2O$). The production cost of phytase is one of the major hindrances in wide spreading of its application. Thus, the utilization of low cost agro-residual waste as a substrate was targeted to obtain high enzyme production (kumar & Sushma, 2012). Gunashree & Venkateswaran (2014); Sandhya *et al.*, (2015) reported rice bran as good substrate for phytase production by *Aspergillus niger*, *Rhizopus oligosporus* and *Aspergillus ficcum.*

During solid-state fermentation, most of the nutrients for the microbial growth are supplied by the substrate. However, the concentration of some nutrients is insufficient in the substrate. It is necessary to provide some supplementation for these inadequate nutrients. In the present study, different nitrogen sources (0.5% w/w) were investigated for their influence on enzyme production. Among various nitrogen sources, NH_4NO_3 at a concentration level of 0.5% (w/w) gave a maximum phytase activity (243.10±5.06 IU/g). Similar results that NH_4NO_3 had the highest phytase production by *Aspergillus niger* and *Rhizopus sp*. were obtained by many researchers (Ramachandran *et al*., 2005; Sandhya *et al*., 2015; Suresh & Radha, 2016).

Among various carbon sources, glucose at 1% (w/w) concentration gave a maximum phytase activity (215.12±5.12 IU/g). Many authors have reported that phytase production was enhanced by glucose in the fermentation medium (Wang *et al*., 2011; Hussin *et al*., 2011; Buddhiwant *et al*., 2016; Sandhya *et al*., 2015). Glucose is considered as a simple carbon source commonly utilized by many microorganisms, leading to an increase in the fungal biomass with a high yield of phytase (Das & Ghosh, 2014; Qasim *et al*., 2017).

The effect of different surfactants on phytase production was tested by supplementing fermentation media with (0.5% w/w) tween-40, tween-80, triton X-100, sodium dodecyl sulphate (SDS) and sodium lauryl sulphate (SLS). The results revealed that at 0.5% concentration, tween-40 gave maximum phytase productivity. Mandviwala & Khire (2000) reported that among various surfactants added to SSF, triton X-100 (0.5% w/w) yielded a 30% increase in phytase productivity, whereas tween-80 yielded a 14% increase by *Aspergillus niger*. Jafari-Tapeh *et al*., (2012); Gupta *et al*., (2014) reported that tween-80 provided maximum phytase production by *Aspergillus niger* NRF9 in solid state fermentation.

The time course of phytase production was studied to determine optimum incubation time required for maximum phytase production. Enzyme production started after 48 h of inoculation and increased with the increase in incubation time up to 120 h, reaching its maximum i.e. (273.08±6.73 IU/g) and afterward, there was a decrease in phytase production. The longer incubation period did not result in the increase of enzyme production due to reduction in nutrients in the substrate, denaturation of the enzyme, catabolic repression or due to production of other by-products (Ramachandran *et al*., 2005; Singh & Satyanarayana, 2006; Wang *et al*., 2011). Phytase production was maximum after 5 days of incubation by *Aspergillus niger* in solid state fermentation as reported by Gupta *et al*., (2014); Gunashree & Venkateswaran (2014); Bakri *et al*., (2018). Previously, 4 days and 6 days were reported as optimum incubation time for phytase production by *Aspergillus niger* and *Aspergillus ficuum*, respectively (Sandhya *et al*., 2015; Tian & Yuan, 2016).

To investigate the influence of incubation temperature on phytase production, the inoculated substrates were incubated at different temperatures i.e. 25, 30, 35, 40, 45 and 50ºC. The results obtained in this study indicated that the optimal temperature for maximum phytase production (275.49±7.36 IU/g) was 35ºC. Hussin *et al*., (2011) found that optimum temperature for *P. stewartii* and *Aspergillus oryzae* SBS50 to produce phytase was 33ºC and 35°C, respectively. Many researchers found that optimum temperature for phytase production is 30ºC by *Aspergillus heteromorphus* MTCC 10685, *Aspergillus niger*, *Aspergillus ficuum* SGA 01, *Aspergillus niger* and *Aspergillus tubingensis* SKA (Lata *et al*., 2013; Gupta *et al*., 2014; Gunashree & Venkateswaran, 2014; Sandhya *et al*., 2015; Suleimenova *et al*., 2016; Qasim *et al*., 2017).

Effect of initial pH (3-9) of culture medium was determined for best phytase production. Maximum phyatse production (271.85±8.41 IU/g) was obtained at initial medium pH 6. Below and above this pH, the

production of enzyme was lower. Earlier related studies reported that pH values ranging from 4.5-6 were optimal for production of phytase by filamentous fungi (Qasim *et al*., 2017). Suleimenova *et al*., (2016) noted that optimum pH for phytase production by *Aspergillus niger* was 5.5. Previous studies also reported that phytase production was optimum at pH 5 by different *Aspergillus* sp., under solid state fermentation (Gupta *et al*., 2014; Singh, 2014; Sandhya *et al*., 2015; Muniz-Marquez *et al*., 2016; Qasim*et al*., 2017; Bakri *et al*., 2018).

Moisture content is one of the most critical factors for microbial growth and enzyme production in solid-state fermentation (Awad *et al*., 2011). The optimum amount of water varies and must be determined for each microbial system (El-Batal & Abdel Karem, 2001). The present study showed that there was linearity between the enzyme production and moisture levels of solid substrate media up to 80%; further increase in the moisture resulted in reduced enzyme production. This may be due to reduced aeration in the substrate and reduced decomposition rate of total organic matter at the lowest and highest moisture contents (Gautam *et al*., 2002; Gunashree & Venkateswaran, 2014). Similar results on the effect of moisture content for phytase production were reported by Bogar *et al*., (2003). Tian & Yuan (2016) observed that the highest enzyme production by *Aspergillus ficum* ATCC66876 using potato wastes was at 77% initial moisture content.

Inoculum size also plays an important role in the extent of growth and metabolite production by fungi. The effect of various inoculum sizes i.e. 5, 10, 15, 20, 25 and 30% (v/w) on phytase production by *Aspergillus niger* was studied. Highest amylase production (297.25±7.94 IU/g) was obtained at 10% (v/w) of inoculum and afterward there was decrease in enzyme production. Similar findings were reported by Gupta *et al*., (2014) obtained maximum phytase yield with 10% inoculum level by *Aspergillus niger* in SSF.

Conclusion

Microbial phytases are having several applications and one of the important applications is to be used as an animal feed supplement, not only to improve phytate digestibility and nutritive value of plant based feed but also to reduce the environmental phosphorus pollution. In the present study, phytase was produced from *Aspergillus niger* using solid state fermentation process, which can be used as a poultry feed additive. Different culture conditions were screened and optimized for the best production of enzyme. The results of this study indicated that optimization of culture conditions are essential for maximum titer of phytase production using solid state fermentation. This is sustainable and a very cost effective way for the production of these useful phytases, and this is the need of today in poultry feed industry.

References

Akturk, S., H.K. Guvenmez and S. Dinçer. 2013. Purification and characterization of extracellular phytase from *Aspergillus niger*. *Curr. Opin. Biotechnol*., 24(Suppl 1) S85.

Awad, G.E.A., M.M.M. Elnashar and E.N. Danial. 2011. Optimization of phytase production by *Penicillium funiculosum* NRC467 under solid state fermentation by using full factorial design. *World Appl. Sci. J.*, 15(11): 1635-1644.

Azeke, M.A., R. Greiner and K.D. Jany. 2011. Purification and characterization of two intracellular phytases from the tempeh fungus *Rhizopus oligosporus*. *J. Food Biochem*., 35: 213-227.

Bakri, Y., H. Ezzeldeen and Y. Akeed. 2018. Screening of phytase producing fungi isolated from Syrian soil. *J. Biol. Innov*., 7(3): 462-474.

Bhavsar, K., P. Buddhiwant, S.K. Soni, D. Depan, S. Sarkar and J.M. Khire. 2013. Phytase isozymes from *Aspergillus niger* NCIM 563 under solid state fermentation: Biochemical characterization and their correlation with submerged phytases. *Proc. Biochem*., 48: 1618-1625.

Bogar, B., G. Szakacs, J.C. Linden, A. Pandey and R.P. Tengerdy. 2003. Optimization of phytase production by solid substrate fermentation. *J. Ind. Microbiol. Biotechnol*., 30(3): 183-189.

Bohn, L., A. Meyer and S. Rasmussen. 2008. Phytate: impact on environment and human nutrition: A challenge for molecular breeding. *J. Zhejiang Univ. Sci*., 9: 165-191.

Buddhiwant, P., K. Bhavsar, R. Kumar and V. Khire. 2016. Phytase production by solid state fermentation of groundnut oil cake by *Aspergillus niger*: a bioprocess optimization study for animal feedstock applications. *Prep. Biochem. Biotechnol*., 46(6): 531-538.

Das, S. and U. Ghosh. 2014. Effect of nutritional supplementation of solid-state fermentation medium on biosynthesis of phytase from *Aspergillus niger* NCIM612. *J. Sci. Ind. Res*., 73: 593-597.

El-Batal, A.I. and H. Abdel Karem. 2001. Phytase production and phytic acid reduction in rape-seed meal by *Aspergillus niger* during solid state fermentation. *Food Res. Int.*, 34(8): 715-720.

Gaind, S. and S. Singh. 2015. Production, purification and characterization of neutral phytase from thermo tolerant *Aspergillus flavus* ITCC 6720, *Int. Biodeterior. Biodegrad*., 99: 15-22.

Gautam, P., A. Sabu, A. Pandey, G. Szakacs and C.R. Soccol. 2002. Microbial production of extra-cellular phytase using polystyrene as inert solid support. *Bioresour. Technol*., 83(3): 229-233.

Gunashree, B. S. and G. Venkateswaran. 2014. Phytase production by *Aspergillus niger* CFR 335 and *Aspergillus ficuum* SGA 01 through submerged and solid- state fermentation. *Sci. World J*., 10: 1-6.

Gupta, R.K., S.S. Gangoliya and N.K. Singh. 2014. Isolation of thermotolerant phytase producing fungi and optimisation of phytase production by *Aspergillus niger* NRF9 in solid state fermentation using response surface methodology. *Biotechnol. Bioproc. Engi*., 19: 996-1004.

Hussin, A.S.M., A.E. Farouk, A.M. Ali and R. Greiner. 2011. Production of phytate-degrading enzyme from Malaysian soil bacteria using rice bran containing media. *J. Agrobiotechnol*., 1: 17-28.

Jafari-Tapeh, H., Z. Hamidi-Esfahani and M.H Azizi. 2012. Culture condition improvement for phytase production in solid state fermentation by *Aspergillus ficuum* using statistical method. *Int. Scholar. Res. Network Chem. Eng*., pp: 1-5.

Kumar, M. and Sushma. 2012. Optimization of production media of novel phytase from *Aspergillus niger* using wheat bran waste. *Int. J. Sci. Res*., 3: 2319-7064.

Lata, S., S. Rostogi, A. Kapoor and M. Imran. 2013. Optimization of culture conditions for the production of phytase from *Aspergillus heteromorphous* MTCC 10685. *Int. J. Adv. Biotechnol. Res*., 4: 224-235.

Maguire, R.O., Z. Dou and J.T. Sims. 2005. Dietary strategies for reduced phosphorus excretion and improved water quality. *J. Environ. Qual*., 34: 2093-2103.

Mandviwala, T.N. and J.M. Khire. 2000. Production of high activity thermostable phytase from thermotolerant *Aspergillus niger* in solid-state fermentation. *J. Ind. Microbiol. Biotechnol*., 24: 237-243.

McKie, V.A. and B.V. McCleary. 2016. A novel and rapid colorimetric method for measuring total phosphorus and phytic acid in foods and animal feeds. *J. AOAC Int.,* 99: 738-743.

Muniz-Marquez, D.B., J.C. Contreras, R. Rodriguez, S.I. Mussatto, J.A. Teixeira and C.N. Aguilar. 2016. Enhancement of fructosyltransferase and fructo-oligosaccharides production by *A. oryzae* DIAMF in solid-state fermentation using aguamiel as culture medium. *Bioresour. Technol*., 213: 276-282.

Neira-Vielma, A.A., C.N. Aguilar, A. Ilyina, J.C. Contreras-Esquivel, G. Michelena-Alvarez and J.L. Martinez-Hernandez. 2018. Purification and biochemical characterization of an *Aspergillus niger* phytase produced by solid-state fermentation using triticale residues as substrate. *Biotechnol. reports*, 17: 49-54.

Omogbenigun, F.O., C.M. Nyachoti and B.A. Slominski. 2003. The effect of supplementing microbial phytase and organic acids to a corn-soybean based diet fed to early weaned pigs. *J. Anim. Sci*., 81: 1806-1813.

Pandey, A., G. Szakacs, C.R. Soccol, J.A. Rodriguez-Leon and V.T. Soccol. 2001. Production, purification and properties of microbial phytases. *Bioresour. Technol*., 77: 203-214.

Qasim, S.S., K.A. Shakir, A.B. Al-Shaibani, and M.K. Walsh. 2017. Optimization of culture conditions to produce phytase from *Aspergillus tubingensis SKA*. *Food Nutri. Sci*., 8: 733-745.

Ramachandran, S., K. Roopesh, K.M. Nampoothiri, G. Szakacs and A. Pandey. 2005. Mixed substrate fermentation for the production of phytase by *Rhizopus spp*. using oil cakes as substrates. *Process Biochem*., 40(5): 1749-1754.

Ramachandran, S., K. Roopesh, K.M. Nampoothiri, G. Szakacs and A. Pandey. 2005. Mixed substrate fermentation for the production of phytase by *Rhizopus spp*. using oilcakes as substrates. *Proc. Biochem*., 40: 1749-1754.

Sandhya, A., A. Sridevi, P.S. Devi and G. Narasimha. 2015. Production and optimization of phytase by *Aspergillus niger. Der Pharmacia Let*., 7(12): 148-153.

Sarita, S., P.K. Sharma and S.D. Chand. 2018. Isolation, identification and molecular characterization of phytase producing thermotolerant fungus, *Aspergillus terreus. Int. J. Sci. Res. Rev*., 7(2): 49-58.

Shahid, M.G. and M. Nadeem. 2015. Screening of *Penicillium* species and optimisation of culture conditions for the production of ergot alkaloids using surface culture fermentation process. *Pak. J. Sci. Ind. Res*., 58(1): 23-29.

Singh, B. and T. Satyanarayana. 2006. Phytase Production by thermophilic mold *Sporotrichum thermophile* in solid-state fermentation and its application in dephytinization of sesame oil cake. *Appl. Biochem. Biotechnol*., 133: 239-250.

Singh, N., A. Kumari, S. Gakhar and B. Singh. 2015. Enhanced cost-effective phytase production by *Aspergillus niger* and its applicability in dephytinization of food ingredients. *Microbiol*., 84: 219-226.

Singh, S.B. 2014. Phytase production by *Aspergillus oryzae* in solid-state fermentation and its application in dephytinization of wheat bran. *Appl. Biochem. Biotechnol*., 173: 1293-1303.

Suleimenova, Z., N. Akhmetsadykov, A. Kalieva, K. Mustafin and Z. Saduyeva. 2016. Effect of different cultural conditions for phytase production by *Aspergillus niger* in submerged fermentation. *Adv. Enz. Res*., 4: 62-67.

Suresh, S. and K.V. Radha. 2016. Statistical optimization and mutagenesis for high level of phytase production by *Rhizopus oligosporus* MTCC 556 under solid state fermentation. *J. Environ. Biol*., 37(2): 253-260.

Thakur, N., P. kumar and D. Chand. 2017. Enhanced production of phytase from thermotolerant *Aspergillus fumigates* isolated from rhizospheric zone of maize fields. *J. Innov. Pharm. Biol. Sci*., 4(3): 114-120.

Tian, M and Q. Yuan. 2016. Optimization of phytase production from potato waste using *Aspergillus ficuum*. *3 Biotech*., 6(256): 1-8.

Wang, Z.H., X.F. Dong, G.Q. Zhang, J.M. Tong, Q. Zhang and S.Z. Xu. 2011. Waste vinegar residue as substrate for phytase production. *Waste Manage Res*., 29(12): 1262-1270.

Yao, M.Z., Y.H. Zhang and W.L. Lu. 2011. Phytases: crystal structures, protein engineering and potential biotechnological applications. *J. Appl. Microbiol*., 112: 1-14.

(Received for publication 11 May 2019)