*Pak. J. Bot.*, 54(6): 2347-2354, 2022. DOI: http://dx.doi.org/10.30848/PJB2022-6(17)

# STRAIN IMPROVEMENT OF *ASPERGILLUS ORYZAE* FOR ENHANCED BIOSYNTHESIS OF PHYTASE THROUGH CHEMICAL MUTAGENESIS

**IKRAM UL HAQ*, MUHAMMAD BILAL SAEED, ALI NAWAZ, ZANNARA MUSTAFA AND FATIMA AKRAM**

*Institute of Industrial Biotechnology, GC University Lahore 54000 Pakistan*
**Corresponding author's email: dr.ikramulhaq@gcu.edu.pk*

**Abstract**

The present study deal with strain improvement through random chemical mutagenesis of phytase producing *Aspergillus oryzae* UJIIB-29. *Aspergillus oryzae* wild strain UJIIB-29 was exposed to three different chemical mutagens i.e. sodium azide, nitrous acid and ethyl methane sulfonate (EMS). Thirty isolates obtained after treating with chemical mutagens showing the large zone of phytic acid hydrolysis were picked and subjected to secondary screening for phytase production using submerged fermentation on phytase screening media. The mutant NA-4 obtained after treating wild strain with nitrous acid exhibited maximum activity of phytase (44± 0.01 U/ml/min). It was approximately 1.9 folds higher than enzyme activity (23.1±0.01 U/ml/min) of parent strain. To further increase the phytase activity, fermentation conditions like fermentation media, time of incubation, incubation temperature, pH of the medium, carbon source and its different concentrations, nitrogen source and its different concentrations and size of inoculum were optimized. The maximum phytase units (58.98± 0.01 U/ml/min) were achieved in glucose phosphate broth (M) having 1% starch, 0.4% ammonium sulphate as a carbon and nitrogen sources, respectively, at 30°C, having pH 4.5 after 7days of incubation period. As a result of optimization, 1.3 times increased in phytase activity (58.98± 0.01 U/ml/min) was achieved. Nitrous acid proved to be a potent mutagen for strain improvement. Furthermore, optimized fermentation parameter can help in scaling up the phytsae production using *Aspergillus oryzae* NA-4.

**Key words:** Mutagenesis; Catalysis; Submerged fermentation, Enxyme, Optimization.

## Introduction

Phytase (EC 3.1.3.8) also known as phosphomonoesterase that hydrolyze the phytic acid in a sequential manner. Phytase releases the myoinositol, inositol phosphate and soluble form of inorganic phosphate (P) (Zeller *et al*., 2015; Li *et al.,* 2021). Based on position of phosphate group ($PO_4^{3-}$) that hydrolyzed first, phytase enzymes are classified into two groups i.e. 3-phytase (E.C. 3.1.3.8) and 6-phytase (E.C. 3.1.3.26). When first phosphate molecule is released from phytic acid, others are also released by step wise action of phytases or acid phosphatases (Haefner *et al*., 2005; Da Silva *et al.*, 2019).

Phytic acid, also called Ins $P_6$ (myo inositol 1, 2, 3, 4, 5, 6 hexakisphoaphate) is major source of organic phosphate in nutrition of animal and storage of phosphate in plants. In cereals, legumens and oilseed crops phytic acid commit approximately 60-90% of total phosphate content (Raboy, 2020). The complex of magnesium, potassium, magnesium and calcium with phytic acid is called phytate that is present in the plants.

Phytase are produced from various animals, microorganisms and plants sources. Because of substrate specificity, high thermostability, catalytic efficiency and low production cost phytases obtain by microbial sources have been targeted in research work (Sabu *et al*., 2002; Dailin *et al*., 2019). Several different filamentous fungal species such as *Aspergillus fumigatus, Aspergillus oryzae, Aspergillus carbonarius* and *Aspegillus niger* are reported for phytase production by fermentation (Casey & Walsh, 2004; Salmon *et al*., 2012). The fungus belongs to *Aspergillus* (genus) yield most suitable and active extracellular enzyme. Therefore, at industrial level for the production of phytase the *Aspergillus* spp. has been preferred (Jatuwong *et al*., 2020 Tanruean *et al.*, 2021).

Microorganisms do not produce the enzyme in a sufficient quantity to cater the needs of industries. Hence, by changing the microorganism's genome, better rate of enzymes production and their characteristics are achieved. To change the genetic material different techniques include recombinant protoplast fusion, recombinant DNA technology and mutation are used (Ushasree *et al.*, 2017). At industrial scale, random mutagenesis for stain improvement plays a very significant role in developing the economical process of microbial fermentation. Two various kinds of mutagenesis such as site direct mutagenesis and random mutagenesis are usually preferred. In the random mutagenesis, physical mutagens and chemical mutagens are favored. Physical mutagens include gamma irradiations, X-rays and ultraviolet irradiations. But, Chemical mutagens includes ethidium bromide, N-methyl N-nitrosoguanidine (MNNG), ethyl methanesulfonate (EMS), nitrous acid, sodium azide and nitrosotrimethyl guanidine (NTG) (Iftikhar *et al*., 2010; Goraya *et al*., 2017; Hassaan *et al*., 2021).

Phytase plays a very significant part in industries belong to animals feed as phytase increases the absorption and digestion of phosphorous contents and several other organic or inorganic nutrients that are insufficiently accessible in diet supplement of several monogastric animals (Vasudevan *et al*., 2017). Phytase has very important role in the biomedical and biotechnological applications because of its anti-inflammatory, antioxidant and anti-cancerous properties. Therefore, in current study random chemical mutagenesis of *A. oryzae* UJIIB-29 was carried out to enhance phytase production.

## Material and Methods

**Fungal strains:** The fungal strain of *A. oryzae* UJIIB-29 that produce the phytase was obtained from IIB (Institute of Industrial Biotechnology), Government College University Lahore. Fungal culture was kept on culture slant of Potato dextrose agar (PDA) and stored in refrigerator at 4°C for further use.

## Mutagenesis

**Spore suspension:** Spore suspension was prepared from 3 days old culture from potato dextrose agar (PDA) slant in 5ml sterile $dH_2O$. The volume of the suspension was raised up to 50ml with 0.01% solution of tween-20. Glass beads was added to break the hyphal mycelia in the spore suspension and placed in shaking incubator for 30min at 30°C. The spore suspension was filtered to remove the fungal mycelia. By serial dilution spore suspension have $10^6$ spores/ml was prepared. Homocytometer was used to count the spores under light microscope (Haq *et al*., 2014).

**Sodium azide treatment:** Took 9ml spore suspension having $10^6$ spore/ml in different test tubes and different concentrations (0.25-2.5%) of sodium azide were mixed up. The reaction was stopped by adding 1ml glycerol (40%) after 30 min. The samples were added in different eppendorfs (Eco Biosciences). The tubes were centrifuged at 4°C and 6000rpm for 15min. Decanted the supernatant and pellet was washed with autoclaved $dH_2O$. Repeat the same procedure for 2-3 times and pellet was re-dissolved in phosphate buffer (Jayaraman & Ilyas, 2010).

**Ethyl methane treatment:** The spore suspension (900ml) containing $10^6$ spores/ml was transferred in various eppendorfs (Eco Biosciences). Different concentrations (10ul- 40ul) of pure Ethyl methane sulfonate were added in eppendorfs (Eco Biosciences). Incubated the reaction mixture at room temperature for 30min. After incubation period, 40% glycerol (500ul) was mixed in the reaction mixture to stop reaction. The reaction mixture was centrifuged at 4°C and 6000 rpm for 15 min. Decanted the supernatant and pellet was washed with sterile phosphate buffer. Repeat the same procedure for 2-3 times (Haq *et al*., 2014).

**Nitrous acid treatment:** Spore suspension (1ml) having $10^6$ spores/ml was added in different test tubes. 1 ml of nitric acid (0.1N) and 1ml of different concentrations (24mM-40mM) of sodium nitrite were mixed up in them. Incubated the reaction mixture in the shaking incubator for 30 min at 30°C and120 rpm. To stop the reaction 40% glycerol (1ml) was added in the reaction mixture. Reaction mixture was centrifuged at 4°C and 6000 rpm for 10 min. The pallet was washed with sterile $d.H_2O$ after supernatant was removed. The same method was repeated thrice (Haq *et al*., 2014).

**Screening of mutant strains:** After treating with chemical mutagens, the suspension of spores was poured and spread aseptically on phytase screening media (PSM) in petri plates. Incubated the plates at 30°C in static incubator for 96 h. Fungal growth pattern was analyzed frequently every 3-4 days. The mutant colonies were selected qualitatively, based upon the diameter of phytic acid hydrolytic zone. The mutant colonies were examined quantitatively by further subjecting them to submerged fermentation for phytase production.

## Enzyme Production

**Preparation of inoculum:** The conidial suspension was prepared from 72h old fungal culture in sterile $dH_2O$. Autoclaved $dH_2O$ (10ml) was added in each slants having profuse conidial growth on its surface. The fungal conidia were scratched aseptically with the aid of inoculating loop. The homogenous suspension of spores was formed by vigorously mixing the solution. The numbers of spores were counted with the help of homocytometer and adjusted as $2.6\times10^6$ spores/ml.

**Submerged fermentation:** Phytase production was carried out in the phytase screening media containing glucose (3g), Ammonium sulphate (1.0g), KCL (0.1g), MgSO4. $7H_2O$ (0.02g), NaCl (0.04g), $CaCl_2$ (0.002g), $FeSO_4.7H_2O$ (0.002g) $MnSO_4$ (0.002g) and sodium phytate (0.25g) in 200ml $d.H_2O$. After sterilization, spore suspension (1ml) was added in the fermentation medium. The fermentation medium was incubated at 30°C and 150 rpm in shaking incubator.

**Extraction of enzyme:** After 7 days (168h) of incubation the cultural broth was filtered with filter paper. After filtration, the filtrate was centrifuged for 10-15 min at 6000rpm. Supernatant was used for estimation of enzyme activity and pellet was discarded.

**Enzyme assay:** Enzyme assay was performed at 50°C in four test tubes named as blank, control, control enzyme and experimental. The reaction mixture (Experimental) had 0.5ml of 0.1M $MgSO_4$ solution, 0.5 ml crude enzyme and 1 ml substrate solution. In control 1ml substrate solution and 0.1M $MgSO_4$ (0.5ml) was added. Control enzyme contained only 0.5ml crude enzyme. Blank contained only 2ml of $d.H_2O$. Test tubes were incubated at 50°C in water bath for 15min. After incubation time, 1ml Trichloro acetic acid (TCA) solution (10%) was added in each test tubes expect control enzyme. Tausky Shorr Color Reagent (1ml) was added in all test tubes expect blank and mixed the control enzyme with control. The absorbance was taken by using the spectrophotometer at 660nm.

One unit phytase activity means, the enzyme quantity requires to liberate one micromole of inorganic phosphate (1umol) in one minute in standard enzyme assay conditions (Racmawati *et al.*, 2017).

$$\text{Units/ml Enzyme} = \frac{(micromoles\ of\ phosphate\ release)\ (df)(5)}{(T)\ (0.5)(2)}$$

where:
df is a dilution factor.
5 is total volume of reaction mixture.
2 volume taken from the reaction mixture.
0.5 is the volume of enzyme.
All the values are measured in ml.

**Optimization of fermentation parameters:** The cultural conditions i.e. fermentation medium, time of incubation, incubation temperature, medium pH, carbon source and its concentration, nitrogen source and its concentration and size of inoculum for phytase production from wild strain (UJIIB-29) and mutant strain (NA-4) of *Aspergillus oryzae* were optimized.

**Statistical analysis**

Three replicates of operational variables were statistically analyzed by using one way ANOVA.

**Results and Discussion**

In order to increase the phytase production from *A. oryzae* UJIIB-29, sixty-two mutant isolates were obtained after exposing fungal spores suspension with various concentrations of nitrous acid, sodium azide and Ethyl methane sulfonate (EMS) for 30min. After qualitative (primary) screening, out of these sixty-two mutant strains, only thirty were subjected to quantitative (secondary) screening because in comparison to wild strain of *Aspergillus oryzae* UJIIB-29 only these 30 mutant strains exhibited the larger zones of phytic acid hydrolysis. Submerged fermentation was used for secondary screening of these 30 mutant strains on PSM (Phytase screening media). Phytase activity by these mutant strains is illustrated in (Fig. 1) (mutagens obtained after sodium azide treatment), (Fig. 2) (mutagens obtained after nitrous acid treatment) and (Fig. 3) (mutagens obtained after EMS treatment). Mutant strain NA-4 obtained after treating with nitrous acid gave maximum phytase activity (44±0.01 U/ml/min), among these 30 mutant strains. 1.9 times increased in phytase activity was achieved in comparison to enzyme activity (23.1±0.01 U/ml/min) by wild strain. Nitrous acid might change the structure of deoxyribonucleic acid (DNA) by substituting the amino group ($NH_2$) with hydroxyl (OH) group at C-6 of the adenine, guanine and cytosine. As result adenine (A) changes into hypoxanthine, guanine (G) changes into xanthine (X) and cytosine (C) changes into uracil (U).

Different media like glucose phosphate broth modified (GPBM), glucose phosphate broth (GPB), glucose peptone media (GPM), peptone potato dextrose broth (PPDB), corn starch media (CSM), Vogel's media (VM), malt extract broth (MEB), malt yeast broth (MYB), czapek dox media (CDM) and phytase screening media (PSM) were used as a fermentation medium for maximum phytase production from both wild strain (UJIIB-29) and mutant strain (NA-4) as shown in (Fig. 4). For both mutant strain (NA-4) and wild strain (UJIIB-29) of *Aspergillus oryzae,* the maximum activity of phytase (57.97± 0.01 U/ml/min and 22 ± 0.01 U/ml/min, prospectively) was obtained on glucose phosphate broth modified. Haq *et al.,* (2020) also used the same composition of glucose phosphate broth for biosynthesis of phytase by *Aspergillus oryzae* and maximum phytase activity (22.3 U/ml/min) was reported at (GPBM). Magnesium sulphate ($MgSO_4.7H_2O$) in the cultural medium acts as a co-factor for enzyme and reduce the permeability of the plasma membrane. Potassium chloride (KCL) in the medium enhances the conidial head formation. Calcium chloride ($CaCl_2$) helps to enhance the branches of hyphae and enhance the uptake of phosphate in the medium. Potassium dihydrogen phosphate maintains the pH for production of phytase by using the $K^+/H^+$ ions channels.

Effect of various incubation period (24h-216h) was examined on yield of phytase by mutant strain (NA-4) and wild strain (UJIIB-29) of *Aspergillus oryzae* as shown in (Fig. 5). The maximum enzyme activity (58.1 ±0.01 U/ml/min, 21.89±0.01 U/ml/min) by mutant strain (NA-4) and wild strain (UJIIB-29), respectively was observed after 168h. Further increase in incubation period after 192h, the phytase activity (16.00 ±0.01 U/ml/min, 54.68 ±0.01 U/ml/min) was decreased from wild strain (UJIIB-29) and mutant strain (NA-4), respectively. Bhavsar *et al.*, (2008) and Haq *et al.*, (2020) also confirmed our wild strain's (UJIIB-29) results that maximum activity of phytase enzyme (22.3 U/ml/min and 68 IU/ml) was obtained after 168h incubation period. In case of mutant strains, Shah *et al.*, (2009), Sing (2013) and Hassaan *et al.*, (2021) reported the maximum phytase activity (80 IU/ml and 2881.60 U/ml) after 14 days, 4 days and 12 days of incubation period, respectively. The differences in results might be because the use of different species of *Aspergillus* and fermentation media for phytase production. The cells of the fungus enter the log phase at very slow rate due to slow consumption of media components.

Different incubation temperatures i.e. 20°C, 30°C, 40°C and 50°C were examined to observe their effect on phytase activity from both wild stain (UJIIB-29) and mutant strain (NA-4) as describe in (Fig. 6). The maximum enzyme activity (22.57± 0.01U/ml/min, 58.4± 0.01 U/ml/min) was achieved by wild strain (UJIIB-29) and mutant strain (NA-4), respectively at 30°C. But in case of mutant a slight decline in activity of phytase enzyme (57.82± 0.01 U/ml/min) was recorded when incubation temperature was 40°C. In case of wild strain (UJIIB-29), Singh (2014), Sandhya *et al.*, (2015) and Haq *et al.*, (2020) published the similar results. But in mutant strain (NA-4), Sapna and sing (2013) and Mahmood *et al.*, (2021) reported that maximum phytase production was achieved at 35°C. The contradictions in the result might be due to the effect of random mutagenesis on fungal strain of *Aspergillus oryzae.* It exhibited that n *Aspergilus oryzae* could be a mesophilic fungus. Incubation temperature effect the microorganism growth as well as the product formation. At very high incubation temperature, metabolic functions of fungus disturbed due to denaturation of proteins and fungal cells starting die.

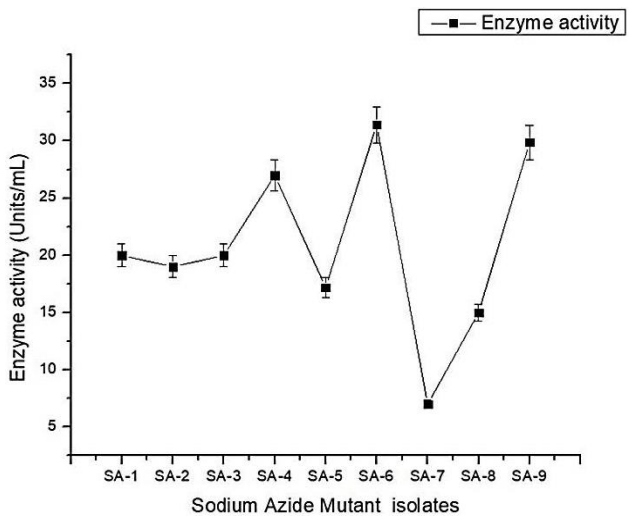


Fig. 1. Production of phytase by Sodium Azide treated mutant isolates. Among three replicates Y-bar showed the standard deviation (S.D. ≤±0.05).

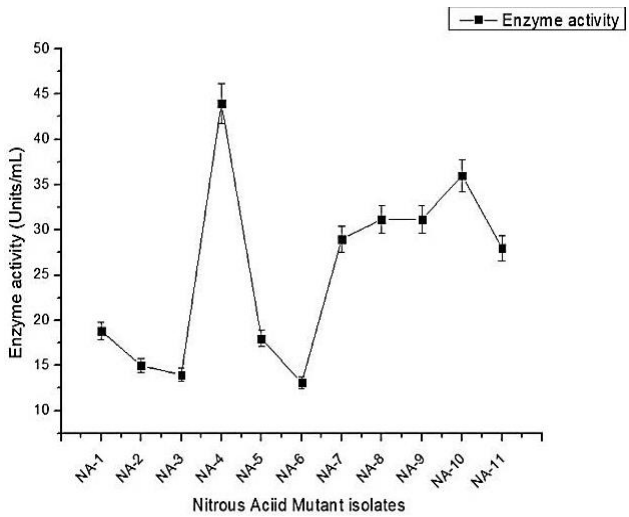


Fig. 2. Production of phytase by Nitrous Acid treated mutant isolates. Among three replicates Y-bar showed the standard deviation (S.D. ≤±0.05).

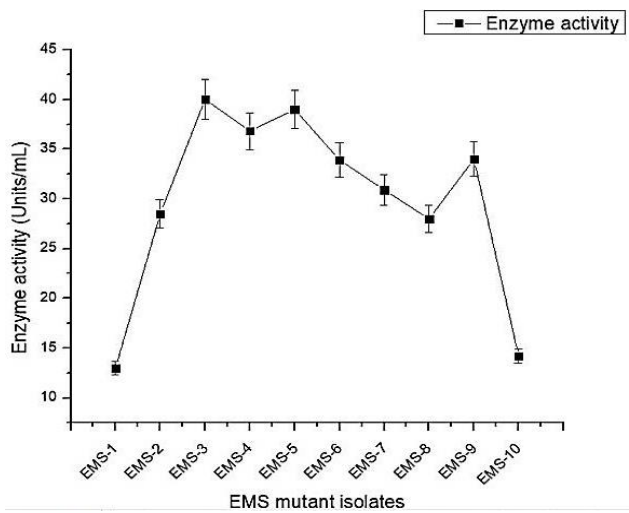


Fig. 3. Production of phytase by EMS treated mutant isolates. Among three replicates Y-bar showed the standard deviation (S.D. ≤±0.05).

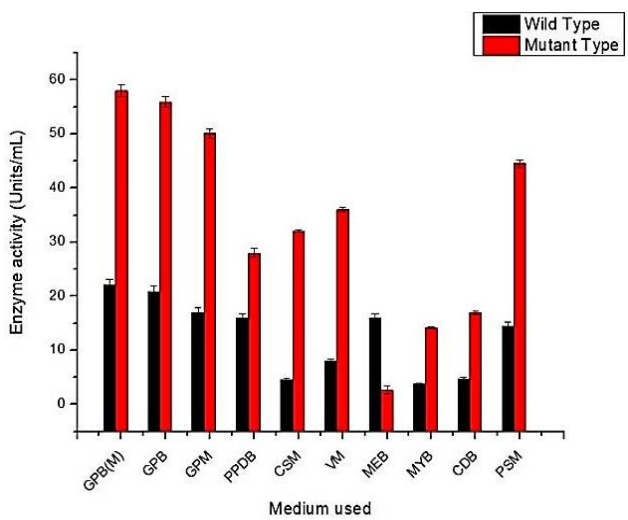


Fig. 4. Effect of different cultural media on phytase activity. Among three replicates Y-bar showed the standard deviation (S.D. ≤±0.05).

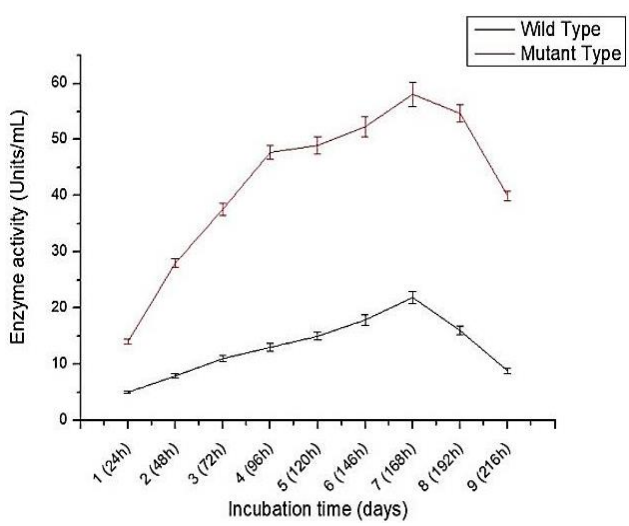


Fig. 5. Effect of incubation time on phytase activity. Among three replicates Y-bar showed the standard deviation (S.D. ≤±0.05).

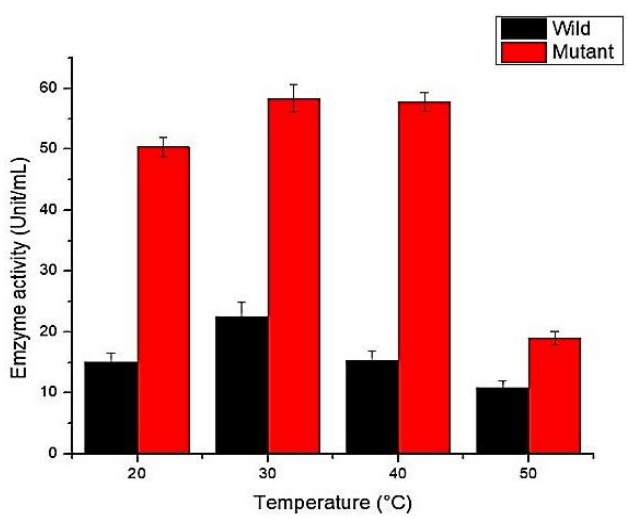


Fig. 6. Effect of incubation temperature on phytase activity. Among three replicates Y-bar showed the standard deviation (S.D. ≤±0.05).

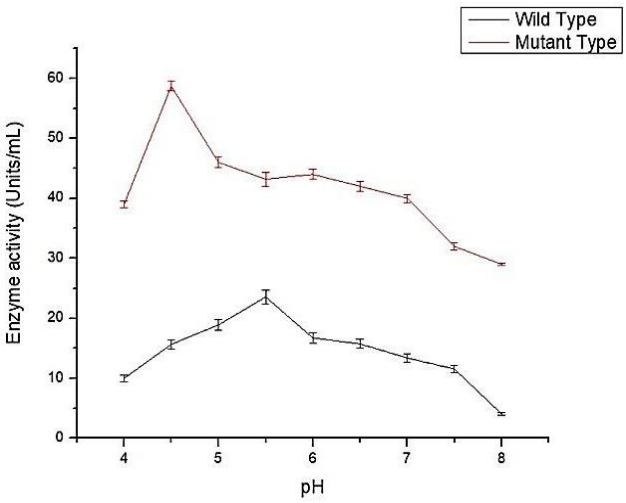


Fig. 7. Effect of pH of fermentation medium on phytase activity. Among three replicates Y-bar showed the standard deviation (S.D. ≤±0.05).

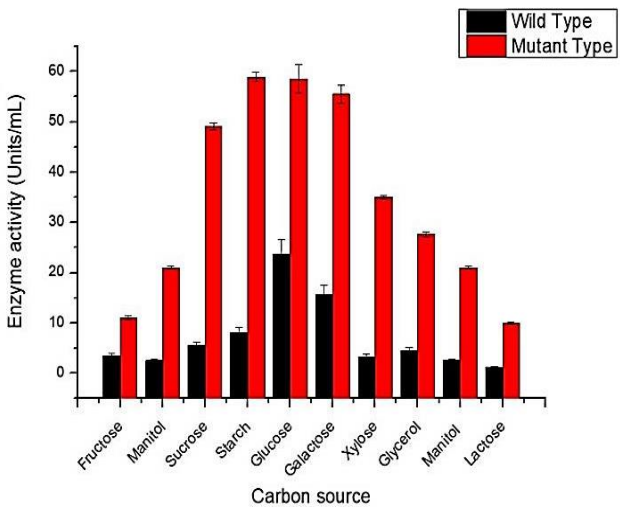


Fig. 8. Effect of different carbon sources on phytase activity. Among three replicates Y-bar showed the standard deviation (S.D. ≤±0.05).

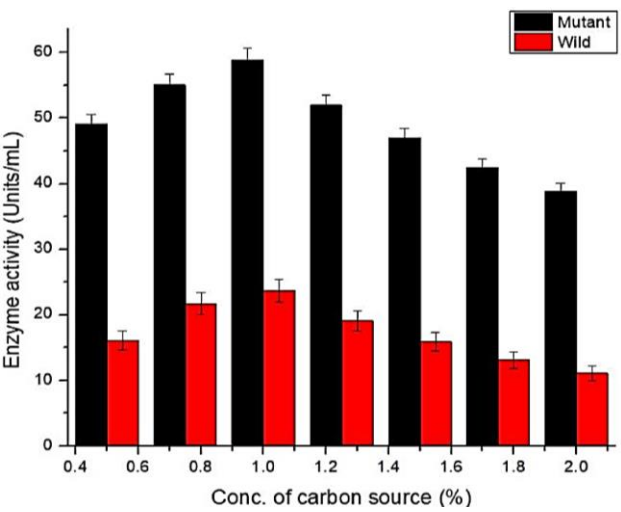


Fig. 9. Effect of different concentrations carbon source on activity. Among three replicates Y-bar showed the standard deviation (S.D. ≤±0.05).

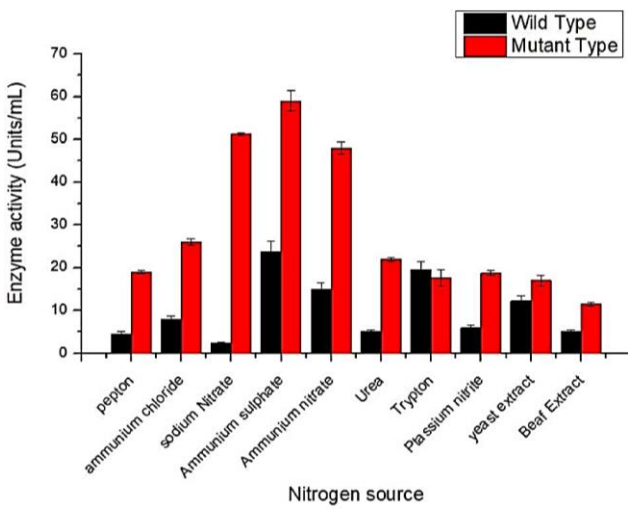


Fig. 10. Effect of different nitrogen sources on phytase activity. Among three replicates Y-bar showed the standard deviation (S.D. ≤±0.05).

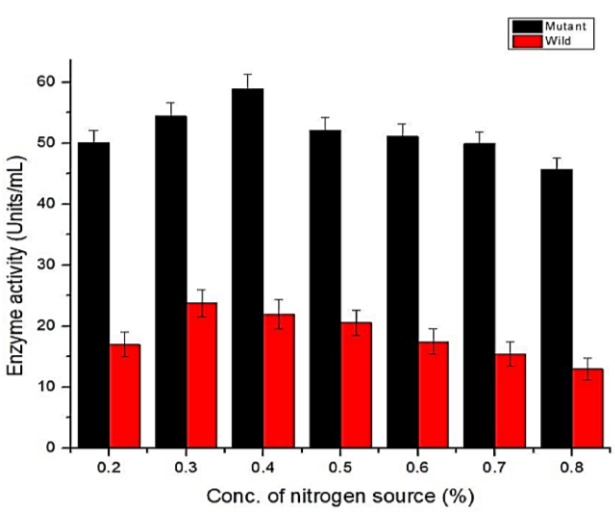


Fig. 11. Effect of different concentrations of nitrogen source on phytase *activity.* Among three replicates Y-bar showed the standard deviation (S.D. ≤±0.05).

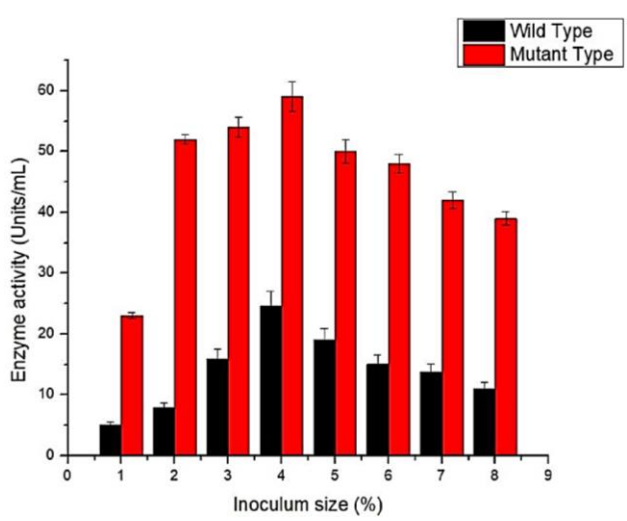


Fig. 12. Effect of inoculum size on phytase production activity. Among three replicates Y-bar showed the standard deviation (S.D. ≤±0.05).

Different pH values of medium (4-8) were examined to check their effect on the phytase production by both wild strain (UJIIB-29) and mutant strain (NA-4) as illustrated in (Fig. 7). At pH 4.5, the maximum activity of phytase (58.7 ± 0.01 U/ml/min) was achieved from mutant strain (NA-4). Shivanna and Venkateswaran (2014) also recorded that maximum enzyme activity was achieved at pH 4.5 in submerged fermentation by *Aspergillus niger.* At 5.5 pH of fermentation medium, maximum activity of phytase enzyme (23.57± 0.01 U/ml/min) was achieved by wild strain (UJIIB-29) of *Aspergillus oryzae*. Haq *et al.*, (2020) and Odeniyi and Itaba (2020) observed that enzyme activity (22.3 U/ml/min and 1 U/ml) was maximum by *Aspergillus oryzae* and *Aspergillus niger* at medium pH 5.5. The presence of high concentration $H^+$ ions effect the permeability of plasma membrane as well as nutrients mobility. The decrease or increase in pH change the amino acids charges and at enzyme active site that decrease the enzyme activity.

Various sources of carbon like glucose, starch, glycerol, xylose, mannitol, fructose, galactose, lactose, sucrose and starch were used to evaluate the production of phytase by both wild strain (UJIIB-29) and mutant strain (NA-4) in fermentation media as exhibited in (Fig. 8). Maximum enzyme activity (23.7 ± 0.01 U/ml/min) from wild strain was attained when glucose was used in the fermentation media. Soni and Khire (2007), Bhavsar *et al.*, (2008) Qasim *et al.*, (2017), Haq *et al.*, (2020) and Mahmood *et al.*, (2021) also recorded the similar results that activity of phytase was highest by *A. niger, A. tubingensis* and *A. oryzae* when glucose was used in the fermentation medium. The maximum phytase units (58.91 ± 0.01 U/ml/min) in case of mutant strain (NA-4) were achieved, when starch (as a carbon source) used in the fermentation medium. The mutant strain (NA-4) showed a slight decrease in phytase activity (58.51±0.01 U/ml/min), when glucose used as a carbon source in fermentation media. Sapna & Singh (2013), Sing (2017) also observed the similar results that production of phytase was maximum from *Aspergillus* ssp. when starch used as source of in the fermentation media.

Different carbon source concentrations (0.5%-2%) were utilized in cultural medium to examine their effect on the phytase activity by mutant strain (NA-4) and wild strain (UJIIB-29) of *Aspergillus oryzae* as described in (Fig. 9). Phytase enzyme activity (23.71± 0.01 U/ml/min, 58.91 ± 0.01 U/ml/min) in case of wild strain (UJIIB-29) and mutant strain (NA-4) was maximum when carbon source (1%) was used in the cultural media. Shah *et al.*, (2009) and Qasim *et al.*, (2017) observed that phytase yield was maximum by suspending glucose as carbon source at concentration 3.5% and 1.5% in cultural media from *A. niger* and *A. tubingensis* respectively. The difference in the results might be because of different species of *Aspergillus* have different metabolical rate. But in case of mutant, Sing (2017) reports the similar results that activity of phytase was highest by *Aspergillus oryzae* when 1% starch was used in the fermentation media.

Different organic and inorganic sources of nitrogen i.e., peptone, sodium nitrite, ammonium chloride, ammonium nitrate, ammonium sulphate, potassium nitrite, urea, beef extract, yeast extract and tryptone were used to evaluate phytase production as shown in (Fig. 10). Both wild strain (UJIIB-29) and mutant strain (NA-4) showed maximum activity of enzyme (23.8 ±0.011 U/ml/min and 58.96± 0.023 U/ml/min, respectively), when ammonium sulphate was used in the cultural media. In case of wild strain (UJIIB-29), Qasim *et al*., (2017), Olubusola & Itaba (2020) and Kumari & Bansal (2021) supported our results that phytase production was maximum when ammonium sulphate was used as a nitrogen source. In case of mutant strain, Ajith *et al.*, (2019) reported that phytase activity was maximum by using the sodium nitrate ($NaNO_3$) in fermentation media. The contradiction might be due to use of different *Aspergillus* spp. like *Aspergillus foetidus* and *Aspergillus oryzae*. Nitrogen is used for amino acid and nucleotides synthesis for microbes. These molecules are the basic units of large polymers such as proteins, deoxyribonucleic acid (DNA) and glucosamine.

Different nitrogen source (ammonium sulphate) concentrations (0.2%-0.8%) were used in fermentation medium to analyze their effect on phytase production by mutant strain (NA-4) and wild strain (UJIIB-29) of *Aspergillus oryzae* (Fig. 11)**.** The mutant strain (NA-4) exhibited the maximum units of enzyme (58.96 ± 0.01 U/ml/min), when 0.4% ammonium sulphate ($(NH_4)_2 SO_4$) concentration was used in fermentation medium. But wild strain (UJIIB-29) gave maximum units of enzyme (23.8± 0.01 U/ml/min), when 0.3% ammonium sulphate ($(NH_4)_2 SO_4$) concentration was used in the fermentation medium. Haq *et al.*, (2020) also observed the similar results that activity of phytase enzyme was maximum by utilizing 0.3% nitrogen source concentration. But in mutant the maximum enzyme activity (58.96± 0.01 U/ml/min) was attained when 0.4% concentration of ammonium sulphate was used in fermentation medium. Shah *et al.*, (2009) recorded that maximum phytase units were attained by using 1% concentration of nitrogen source in the fermentation media. It might be because to use different *Aspergillus* species like *Aspergillus niger* and *Aspergillus oryzae.*

Size of inoculum is another significant factor that plays a significant role for enzyme production. The inoculum size was varied (1-8%) to evaluate its effect on phytase production by wild strain (UJIIB-29) and mutant strain (NA-4) as exhibited in (Fig. 12). In both wild strain (UJIIB-29) and mutant strain (NA-4), 4% inoculum size showed maximum yield of enzyme (24.6± 0.01U/ml/min and 58.98± 0.01 U/ml/min, respectively). In wild strain (UJIIB-29), Vassilev *et al.*, (2007) and Haq *et al*., (2020) also recorded the same results of phytase production from the *Aspergillus niger* and *Aspergillus oryzae.* But in mutant strain, shah *et al.*, (2009) use the 1% inoculum size to achieve maximum production of phytase after mutagenesis of *A oryzae.* The contradiction in results might be because of effect of chemical mutagens during the random mutagenesis and use of different fermentation media.

## Conclusion

*Aspergillus oryzae* mutant strain NA-4 was acquired as a promising microorganism for phytase biosynthesis that produced 1.9 times higher activity of phytase as compared to wild strain of *Aspergillus oryzae.* Optimization of various fermentation conditions highly influenced the phytase production. 1.3 times enhance in production of phytase was attained after optimization of various fermentation conditions. The recent study will aid to make an economical and effective biochemical process for biosynthesis of phytase at large scale. The process can be utilized in the poultry feed industry and have key significance for human ecologists and nutritionists.

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(Received for publication 20 April 2021)