



## Original article

Physical and chemical mutagens improved *Sporotrichum thermophile*, strain ST20 for enhanced Phytase activity

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## ABSTRACT

**Objective:** Phosphorous is an essential micronutrient of plants and involved in critical biological functions. In nature, phosphorous is mostly present in immobilized inorganic mineral and in the fixed organic form including phytic acid and phosphoesteric compounds. However, the bioavailability of bound phosphorous could be enhanced by the use of phosphate solubilizing microorganisms such as bacteria and fungi. The phytases are widespread in an environment and have been isolated from different sources comprising bacteria and fungi.

**Methodology:** In current studies, we show the successful use of gamma rays and EMS (Ethyl Methane Sulphonate) mutagenesis for enhanced activity of phytases in a fungal strain *Sporotrichum thermophile*.

**Results:** We report an improved strain ST2 that could produce a clear halo zone around the colony, up to 24 mm. The maximum enzymatic activity was found of 382 U/mL on pH 5.5. However, the phytase activity was improved to 387 U/ml at 45 °C. We also report that the mutants produced through EMS showed the greater potential for phytase production.

**Conclusion:** The current study highlights the potential of EMS mutagenesis for strain improvement over physical mutagens.

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## 1. Introduction

Phosphorous is an essential micronutrient for living organisms, required for their growth, reproduction, and metabolism. Despite

present in abundance in our ecosystem, its bioavailability to plants, animals, and microbes in free or solubilized form is limited due to slowest recycling of bound phosphorous (Filippelli, 2002; Oelkers et al., 2008). In nature, phosphorous is mostly present in an immobilized inorganic mineral form such as orthophosphate and apatite, and in the fixed organic form including phytic acid (present in food of plant origin) and phosphoesteric compounds (Ashraf et al., 2013). The release of bound phosphorous from minerals during geochemical processes is limiting and depends mainly upon weathering (Filippelli, 2002). Another mechanism of increasing the bioavailability of bound phosphorous is through biotic means including the use of phosphate solubilizing microorganisms such as bacteria and fungi. Conversely, the microbes release a variety of phosphate solubilizing enzymes known as phytases which cat-

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alyze the hydrolysis of phytic acids and phosphoester bonds to release free inorganic phosphate (Ashraf et al., 2013; Rasul et al., 2019; Zaheer et al., 2016, 2019). The recycling of immobilized phosphorous through biological means is gaining more attention over artificial fortification of soil with phosphate fertilizers (Qvirist et al., 2017). Especially, the phytases mediated hydrolysis of phytic acids is a critical step in the cycle of biogeochemical phosphorous (George et al., 2007).

Phytases have been isolated from different sources like plants, animals, bacteria, fungi (Sajidan et al., 2004). Microbial phytases, in particular from fungi, have been used as additives in animal feed and as plant growth promoters due to their higher bioefficacy of phytate degradation than cereal phytases (Zimmermann et al., 2002). The first commercial phytase supplemented animal feed, introduced in the market in 1991, was of fungal origin obtained from *Aspergillus niger* var. *ficuum* (Simon and Igbasan, 2002). It was sold under the trade name Natuphos in Europe (Misset, 2003). Currently, all authorized phytase preparations sold in European Union is basically derived from different genetically engineered strains of filamentous fungi (Haefner et al., 2005). The use of phytase additive feed has great nutritional benefits for livestock as monogastric animals could not efficiently extract phosphorous from fodder (Greiner and Konietzny, 2006). Due to the high commercial importance of phytases, different optimization strategies have been described in the literature to increase its yield from microbial cultures (Qvirist et al., 2017).

The present work concerns the use of two different mutagenesis methods for strain improvement of phytase producing locally isolated thermophilic mold, *Sporotrichum thermophile*. Moreover, the study deals with the optimization of the fungal culture conditions to enhance its phytate degradation capacity by increasing phytase production efficiency.

## 2. Methodology

### 2.1. Isolation of phytate from fungus

Fungal strains were isolated from the plant by serial dilution. Phytate specific medium containing 1.5 g Dextrose, 0.5 g  $\text{NH}_4\text{NO}_3$ , 0.05 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.001 g  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 0.05 g KCl, 0.001 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g of 1% Sodium Phytate, 2 g Agar and distilled water to make the volume 100 ml. pH was adjusted to 5.5 by adding NaOH. Colonies which were capable to hydrolyze sodium phytate by clear halo zone in their surroundings were obtained and purified by sub-culturing on PDA (potato dextrose agar) plate (Rodríguez and Fraga, 1999).

### 2.2. Qualitative screening

Qualitative screening assay of fungal strains using PDA in Petri plates; Halo zone and colony diameters on Petri-plates containing PDA media were measured from 3 to 14 days. Plates were incubated at 28 °C (Lan et al., 2002).

### 2.3. Quantitative screening

Quantitative screening assay of fungal strains using 50 ml of PDA media will be performed according to the procedure described in the literature (Rodríguez and Fraga, 1999).

### 2.4. Optimization of culture condition

For determination of the optimal growth conditions and phytase production; the temperature and pH range were used from

20 °C to 70 °C and pH 3.5 to 8.5 respectively, according to previously described (Jorquera et al., 2008).

### 2.5. Effect of incubation temperature

Incubation temperature was adjusted from 20 °C to 70 °C with an interval of 10 °C for five days to optimize maximum phytase production.

### 2.6. Effect of pH

Media pH was adjusted from 3 to 9 with an interval of 0.5 with HCl or NaOH to check the effect of pH on phytase production.

### 2.7. Sample collection

The strain of *Sporotrichum thermophile* was collected from National Institute for Biotechnology and Genetic Engineering collection center under contamination-free conditions in sterilized falcon tubes.

### 2.8. Treatment with physical mutagenesis

#### 2.8.1. Gamma radiations

To improve the phytase quality and production of *Sporotrichum thermophile*, Mark-IV irradiator was used to expose the strain with gamma radiations available at Nuclear Institute for Agriculture and Biology, Faisalabad. The grown cultures of the strain on slants were scrapped off with sterile distilled water and diluted with sterile saline containing 0.1% Tween20. The 80.5 ml of cell culture transferred to every single vessel was subjected to gamma irradiator. The various doses of gamma radiations chosen for current experiments were 20, 40, 60, 80, 100, 120, 140 160 and 180 kilorads.

#### 2.8.2. Screening medium for growth of mutant strains

For the preparation of 100 ml media, 1.5 g Dextrose, 0.5 g  $\text{NH}_4\text{NO}_3$ , 0.001 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.05 g KCl, 0.001 g  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 0.5 g of 1% Sodium Phytate, 0.05 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 50  $\mu\text{l}$  Triton X-100 were liquefied in 100 ml distilled water. The pH of the medium was maintained at 5.5 by adding NaOH.

#### 2.8.3. Sample spreading

Total 10  $\mu\text{l}$  of samples were taken, vortex and poured on solidified medium plate with the help of pipette and were spread with the help of disposable loop. Petri plates were hatched at 45 °C and their growth was assessed after 16 h.

#### 2.8.4. Selection of mutants

For best results, Triton x-100 (0.05%) was consumed in PSM media to limit the formation of fungus.

#### 2.8.5. Plate screening procedure

Phytase screening medium along with Triton x-100 (basal media) was used for choosing mutant. Subsequently two to three days of incubation at 45 °C in black, the magnitude of clearance zone was dogged. The colonies displaying larger zones were further sub-cultured. A small number of colonies were found displaying bigger clearing zones than wild type (Jorquera et al., 2008).

#### 2.8.6. Spore inoculum

For spore inoculation 5–7 days old cell suspension was taken. Disinfected injecting needle was used to scuff the dormant spores.

### 2.8.7. Vegetative inoculum

For the preparation of vegetative inoculum, 1000 ml of PSM (phytase screening medium) media g/L (glucose 30 g, NaNO<sub>3</sub> 8 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5 g, KCl 0.5 g, FeSO<sub>4</sub>·7H<sub>2</sub>O 0.01 g, KH<sub>2</sub>PO<sub>4</sub>·7H<sub>2</sub>O 0.01 g, pH5.5) having glass bead in a conical flask of 1 l sealed with a cotton plug was autoclaved at 121 °C, 15 lb/inch<sup>2</sup> pressure for 15 min. 1 ml of fungal culture (comprising 4.63 × 10<sup>7</sup> spores) was aseptically shifted to the conical flask. The conical flask was placed in an incubator shaker at 200 rpm, 30 °C for 24 h (Iftikhar et al., 2010).

The chosen mutant isolates along with wild type were compared. About 50 ml of fermentation media was transferred to every single Erlenmeyer flask. The Erlenmeyer flasks were incubated in the orbital shaker at 30 °C with 200 rpm. The flask contents were used for evaluation of enzyme activity. All the experiments were carried out in three replications (Iftikhar et al., 2010).

### 2.9. Treatment of strain with chemical mutagenesis

#### 2.9.1. Ethyl methane Sulphonate treatment

The spore suspension of mutant (SPUV002) was grown on phytase screening medium for 5 days at 28 °C. Preparation of spore suspension in phosphate buffer was similar to that described in gamma radiation treatment. Five milliliters of spore suspension was added to 15 ml of Ethyl Methane Sulphonate solution (3 mg/ml) and kept on a rotary shaker at 28 °C for 120 min. for the reaction to proceed. During this period, 2 ml aliquots were taken out at an interval of 30, 60, 90, 120 and 150 min. The aliquots were pallet down by centrifugation and pellets were washed three times with sterile distilled water. Subsequently, the pellets were re-suspended in sterile phosphate buffer to stop the reaction. The serially diluted samples in the same phosphate buffer were spread on phytase screening medium. Further, the plates were incubated for 5 days at room temperature.

### 2.10. Phytase assay

A phytase assay was used to examine the total phytase activity of crude enzyme extracts as a previously described method (Engelen et al., 1994).

### 2.11. Statistical analysis

All data represented as the mean of three replicates and their standard deviation.

## 3. Results

### 3.1. The strain ST2 showed promising halo zone on phytase screening medium

For isolation of phytase producing fungal strains, we used PSM containing sodium phytate. On the origin of apparent halo zone, 7 best fungal strains were remote on phytase-specific medium (Fig. 1A). The best strain ST2 was found and produced approximately 24 mm apparent halo zone about the colony on PDA medium (Fig. 1B).

### 3.2. Optimization of culture condition of isolated strain ST2

Carbon sources, Nitrogen sources, pH and temperature were those parameters which were used for the optimization of culture conditions.

### 3.3. Strain ST2 showed the highest phytase activity on dextrose carbon source

Strain ST2 was grown on various sugars like glucose, maltose, lactose, sucrose, dextrose and fructose to investigate the best carbon source for phytase activity. It was found that dextrose was the best carbon source where strain ST2 showed highest phytase activity of 381 U/mL on the 3rd day (Table 1).

### 3.4. Strain ST2 showed highest phytase activity on pH 5.5

In different flasks, pH gradients were taken from 3.5 to 8.5. The strain ST2 showed maximum enzyme activity of 382 U/mL on pH 5.5 (Table 2).

### 3.5. Yeast extract as nitrogen source yielded maximum phytase activity

To investigate the effect of nitrogen source on phytase activity, ammonium acetate, ammonium sulfate, sodium nitrate, urea and yeast extract was used as nitrogen sources. On yeast extract strain ST2 showed phytase activity was 395 U/mL (Table 3).

### 3.6. Temperature effect on enzyme activity of strain ST2

The strain was cultured on various temperatures ranging from 20 °C to 70 °C to check the effect of temperature on enzyme activ-

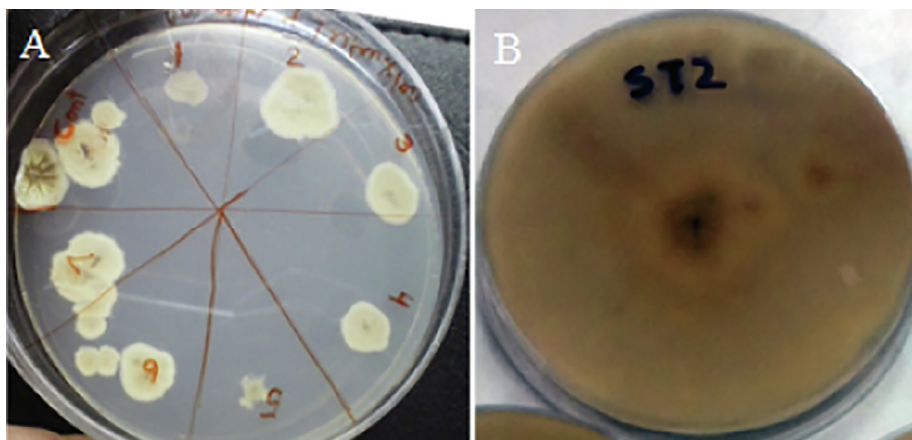


Fig. 1. (A) Phytase producing fungal strains on phytase screening medium, (B) Strain ST2 of *Sporotrichum thermophile* forming halo zone on potato dextrose agar medium.

**Table 1**  
Effect of different carbon sources on phytase activity of strain ST2.

Carbon Source	Enzyme Activity, U/mL (Days)				
	1	2	3	4	5
1% glucose	169 ± 0.87	286 ± 2.41	347 ± 1.63	302 ± 1.6	254 ± 1.43
Sucrose	198 ± 2.67	256 ± 1.34	312 ± 2.34	315 ± 2.78	269 ± 1.56
Fructose	205 ± 2.23	276 ± 2.89	326 ± 2.98	338 ± 1.67	246 ± 2.77
Maltose	212 ± 1.23	298 ± 1.54	345 ± 1.45	347 ± 1.79	259 ± 1.30
Lactose	236 ± 1.65	265 ± 2.32	329 ± 1.96	310 ± 2.67	279 ± 1.12
Glucose + Sucrose	256 ± 1.12	299 ± 1.10	334 ± 1.12	328 ± 1.74	312 ± 1.32
Dextrose	278 ± 1.21	311 ± 1.54	381 ± 1.12	320 ± 2.99	278 ± 1.23
Glucose + Lactose	289 ± 2.34	300 ± 1.23	339 ± 1.23	318 ± 1.33	301 ± 1.12

**Table 2**  
Optimization of pH for maximum enzyme activity of strain ST2.

Days	pH (Enzyme activity U/mL)					
	3.5	4.5	5.5	6.5	7.5	8.5
1	47 ± 1.33	76 ± 1.3	156 ± 1.34	86 ± 1.61	52 ± 1.53	39 ± 1.42
2	84 ± 2.53	113 ± 1.62	248 ± 2.22	139 ± 2.32	98 ± 1.32	72 ± 2.12
3	95 ± 1.90	156 ± 1.22	382 ± 1.53	169 ± 1.92	154 ± 2.78	90 ± 1.34
4	109 ± 2.39	179 ± 1.98	321 ± 1.52	179 ± 1.12	169 ± 2.34	189 ± 2.99
5	198 ± 1.03	201 ± 1.99	299 ± 1.53	199 ± 1.23	265 ± 1.99	299 ± 1.45

**Table 3**  
Screening of various nitrogen sources for maximum phytase activity of strain ST2.

Days	Source of Nitrogen				
	Urea	NaNO <sub>3</sub>	Yeast Extract	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	CH <sub>3</sub> COONH <sub>4</sub>
1	193 ± 1.72	64 ± 1.39	180 ± 1.72	187 ± 1.62	163 ± 1.23
2	201 ± 4.93	194 ± 2.34	185 ± 2.91	211 ± 1.90	198 ± 2.23
3	286 ± 8.23	265 ± 1.12	395 ± 2.54	301 ± 2.45	299 ± 2.12
4	235 ± 8.11	204 ± 2.18	302 ± 2.45	312 ± 1.76	275 ± 2.99
5	278 ± 6.27	322 ± 1.85	342 ± 2.43	289 ± 2.49	265 ± 2.23

**Table 4**  
Effect of temperature on enzyme activity of strain ST2.

Temperature (°C)	Activity, U/mL
20	79 ± 1.71
25	190 ± 1.67
35	223 ± 1.11
45	387 ± 1.12
55	321 ± 1.11
65	354 ± 1.43

ity. It was observed that at 45 °C, strain ST2 showed the enzymatic activity of 387 U/ml (Table 4).

### 3.7. Strain improvement by using Gamma radiations as physical mutagenesis

A culture of 10 µl was exposed to various experimented doses of gamma radiation i.e., 20, 40, 60, 80, 100, 120, 140 160 and 180kilo-rads. The treated culture of *Sporotrichum thermophile* was spread on the PSM plates in the incubator at 45 °C and colonies were appeared in the plates (Fig. 2). The sample treated with 20Kd showed 300 approx. colonies of *Sporotrichum thermophile* whereas sample with a dose of 40Kd, 60Kd, 80Kd, 100Kd, 120Kd, 140Kd, 160kd, and 180Kd showed approximately 122, 102, 43, 40, 35, 30, 28, 12 colonies of *Sporotrichum thermophile* respectively (Table 5).

### 3.8. Kill curve for physical mutagenesis

Kill curve calculation is the basic step of mutagenesis experiments. It helps researchers 'determine the concentration or time

period of exposure of the mutagenic agent for the mutagenesis to succeed. The time interval for a 99% kill was obtained (Fig. 3).

### 3.9. Identification of colonies and mutant selection

Picked out the colonies from each plate which showed prominent growth then these colonies were added on the PSM plates and incubated them at 45 °C (Fig. 4). Those identified colonies which showed accurate growth and clearance. Picked them separately on other PSM plates for the sake of finding the potential mutants (Fig. 5).

### 3.10. Mutant STM6 showed enhanced enzymatic activity than the parent strain

The enzymatic activity of the cultures was measured. Mutant STM6 was found to be superior to the parent strain. STM6 mutant isolated showed an increase in activity up to 17,931 U/1/d, with the increase in productivity up to 2.9 times that of parent respectively (Table 6).

In Gamma radiation treatment mutant STM6 showed the highest fermentation activity on the 5th day as compared to other mutants (Fig. 6).

### 3.11. Strain improvement by using EMS as chemical mutagenesis

To investigate the efficiency of chemical mutagenesis, 5 ml of spore suspension was treated with 15 ml of EMS solution (3 mg/ml) for 5 times. Serial dilution of the samples was done and spread on PSM medium plated for colonies appearance (Table 7).

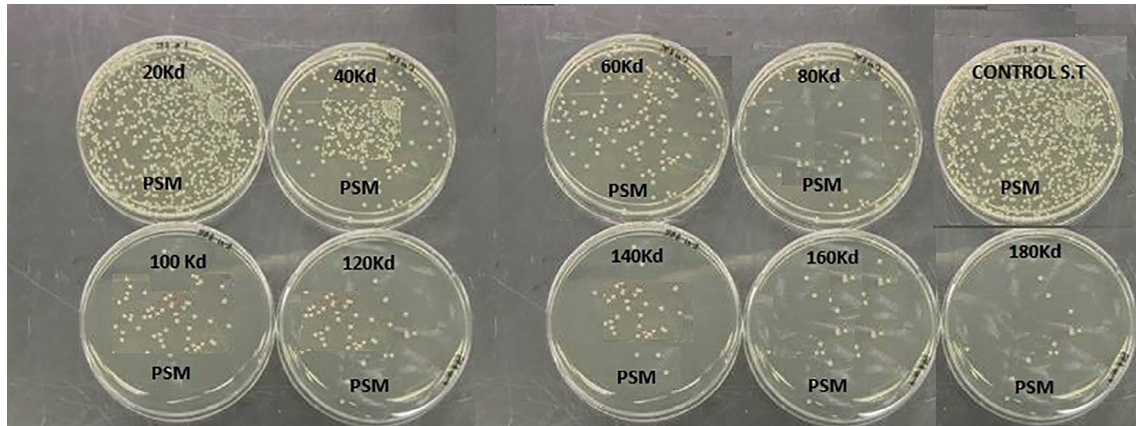


Fig. 2. Showing approximate number of colonies of *Sporotrichum thermophile* after different doses of gamma radiations in kilorads.

**Table 5**  
Number of colonies of *Sporotrichum thermophile* at different concentrations of gamma radiation doses.

Gamma Radiation Kilorads	CFU/ml (colony forming units)	Log (CFU/ml)
180	12 ± 1.12	4.079
160	28 ± 1.52	4.447
140	30 ± 2.93	4.612
120	35 ± 1.26	4.801
100	40 ± 1.62	4.902
80	43 ± 1.21	5.613
60	102 ± 1.80	7.008
40	122 ± 1.94	7.086
20	300 ± 2.63	7.577

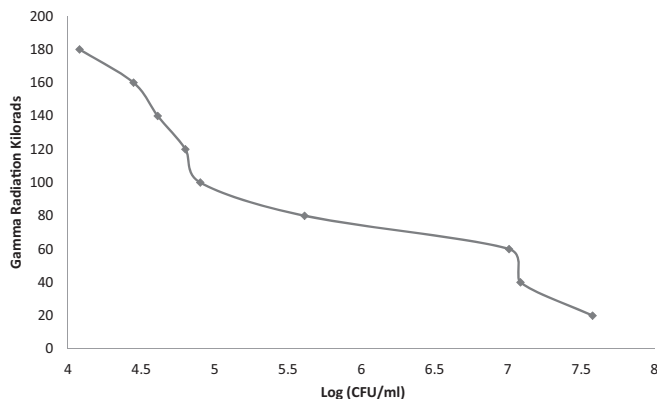


Fig. 3. Survival curve of colonies of *Sporotrichum thermophile* at different concentrations of gamma radiations.

### 3.12. Survival of the strain decreased with an increase in EMS treatment time

Survival curve of colonies of *Sporotrichum thermophile* at the time interval of EMS for 99% kill was obtained (Fig. 7). It indicated an inversely proportional relationship between EMS reaction time and survival rate.

### 3.13. STM6 mutant increased phytase production than the parent strain

The phytase and production of various isolated mutants were measured. It was found that mutant STM6 showed an increase in activity up to 19,875 U/1/d, with an increase in productivity up

to 3.2 times that of the parent (Table 8). Further, in ethyl methane Sulphonate treatment-generated mutant STM6 showed the highest fermentation activity on the 5th day as compared to other mutants (Fig. 8).

## 4. Discussion

Phosphorous is an essential micronutrient for plants, involved in key biological mechanisms including growth, reproduction, and metabolism. Although it is abundantly present nature, its bioavailability to plants in free or solubilized form is limited due to slowest recycling of bound phosphorous (Filippelli, 2002; Oelkers et al., 2008). However, there are a variety of phosphate solubilizing enzymes known as phytases released by microbes which catalyze the release of free inorganic phosphate. Phytases are widespread in the environment and have been isolated from different sources like animals, bacteria, fungi, and plants (Ashraf et al., 2013; Rasul et al., 2019; Sajidan et al., 2004; Zaheer et al., 2016; Zaheer et al., 2019). Though several organisms have been reported to produce phytase, the expression levels are far from the idea of commercialization with an economical point of view. *Sporotrichum thermophile* is a fungus extensively used in the investigation for the strain improvement. In current studies, combinatorial mutagenesis approach was adopted using different combinations of ethyl methane sulphonate with that of gamma radiations exposure, for obtaining potential mutant strains. The best mutant who showed apparent halo region was selected on phytase screening medium.

In the present studies, we isolated 7 fungal strains of *Sporotrichum thermophile*. For optimization of phytase production, the best strain showed the highest phytase activity was chosen. The best fungal strain ST2 isolate which was achieved with an optimized process showed optimum productivity of phytase. The parameters used for optimization such as carbon sources, nitrogen sources, incubation temperature, initial pH, and incubation period in days.

For optimization of culture conditions e.g., pH and temperature. pH gradients were used from 3.5 to 8.5 in different flasks. This strain illustrated the enzymatic activity of 382 U/ml at pH 5.5. Similarly for the optimization of other parameters used such as Carbon source, Nitrogen source. Carbon sources like 0.75% of different disaccharides, glucose, maltose, lactose, sucrose, dextrose, and fructose used. Strain demonstrated best phytase activity of 381 U/ml on dextrose. Nitrogen sources i.e., yeast extract, sodium nitrate, ammonium sulfate, urea and ammonium acetate were used. Among all these sources, phytase activity was 395 U/ml on yeast extract. Temperature parameter was experienced that is 20 °C to 70 °C. At 45 °C, the strain showed maximum enzyme activity of 387 U/ml phytase activity.

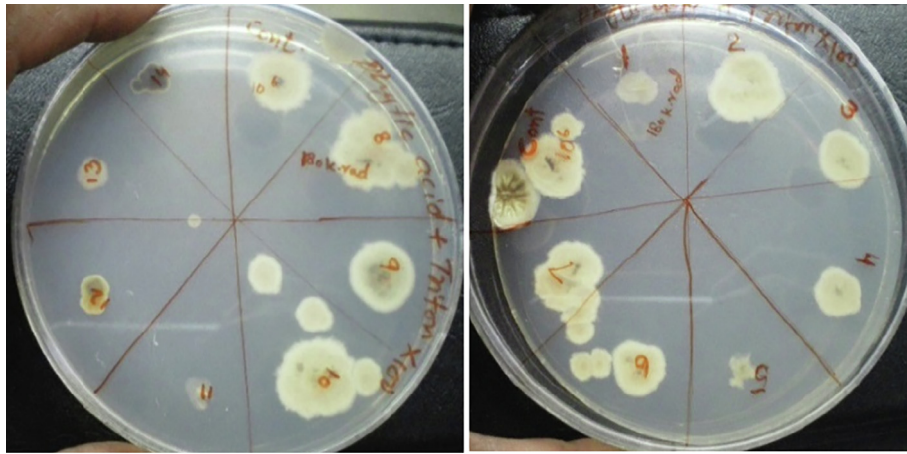


Fig. 4. Growth and clearance of all picked colonies.

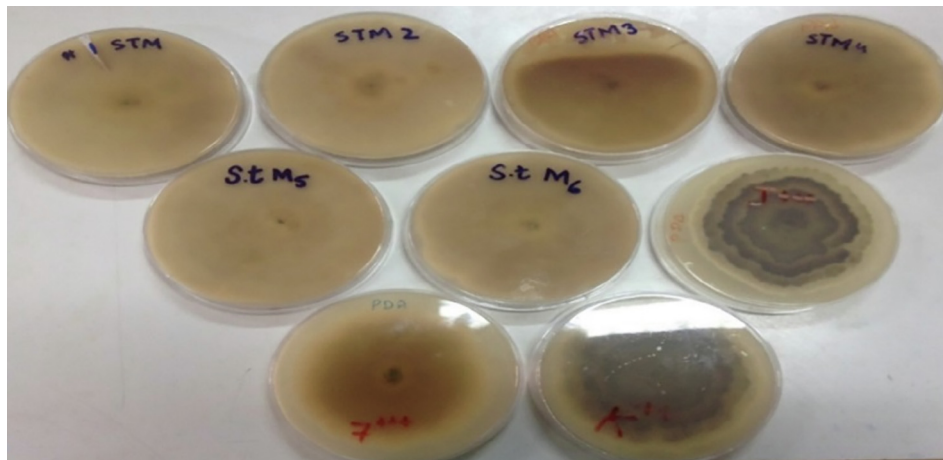


Fig. 5. Selected potential mutants of *Sporotrichum thermophile* for phytase production.

**Table 6**  
Comparison of phytase production between parent and mutant strains.

Strain	Productivity folds	
	(U/l/d)	Increase
Parent	6183 ± 2.83	1
STM-1	14221 ± 2.21	2.3
STM-2	16694 ± 2.15	2.7
STM-3	13603 ± 2.41	2.2
STM-4	14839 ± 2.25	2.4
STM-5	15458 ± 2.43	2.5
STM-6	17931 ± 2.71	2.9

The above method is not able to distinguish among phytase action and acid production. Thus, statistically, optimization occurs for the parental strain, shake flask method used and confirmed the phytase production by all quantify positive mutants. In Physical mutagenesis amongst the good STM6 mutant, the mutant of *Sporotrichum thermophile* exhibit the highest phytase productivity (17,931 U/l/d) and enhanced 2.9 folds yield as compared to the productivity obtained from parent strain. These observations might have wonderful value addition to phytate feed-conversion and ecological aspect of phytate biology.

Strain improvement has its own pros and cons. Yet, there is no assurance that the mutants with preferred phenotype showed the mutation in the gene of interest also low mutation frequency in the desired gene is another difficulty associated with strain improve-

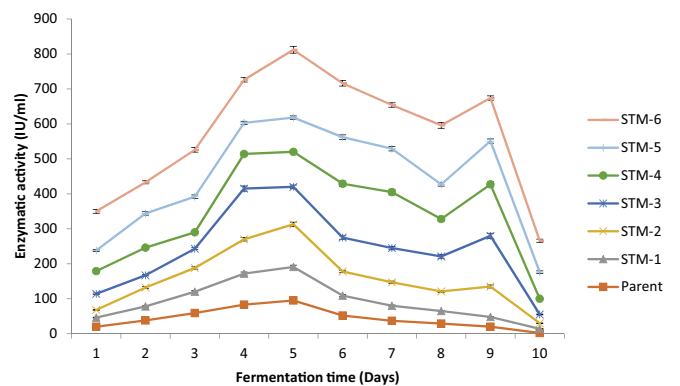


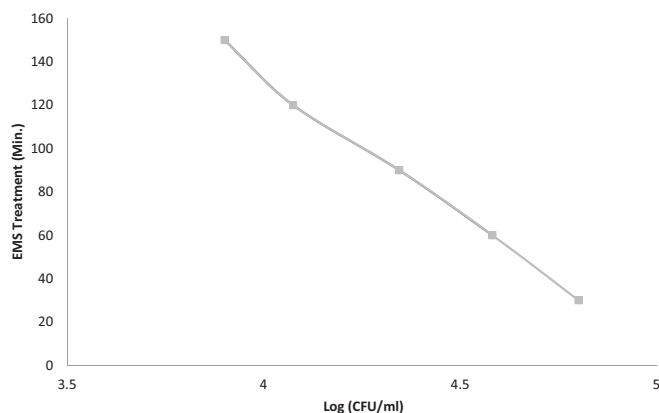
Fig. 6. Activity profile of mutant strain by gamma radiations under fermentation condition.

ment. In chemical mutagenesis, STM6 mutant isolated showed an increase in activity up to 19,875 U/l/d, with an increase in productivity up to 3.2 times that of the parent. Overcoming these shortcomings and exploitation of recent developments in gene cloning and recombination can be applied to study the site and type of mutagenesis. Though in mutagenesis studies morphological differences were seen we can logically suppose that genetic changeability between parent and mutant strains can exist.

**Table 7**

Number of colonies of *Sporotrichum thermophile* at different time intervals of EMS treatment.

EMS Treatment (Min.)	CFU/ml	Log (CFU/ml)
150	10 ± 1.61	3.901
120	11 ± 1.50	4.075
90	26 ± 1.15	4.345
60	28 ± 1.19	4.582
30	34 ± 1.44	4.801

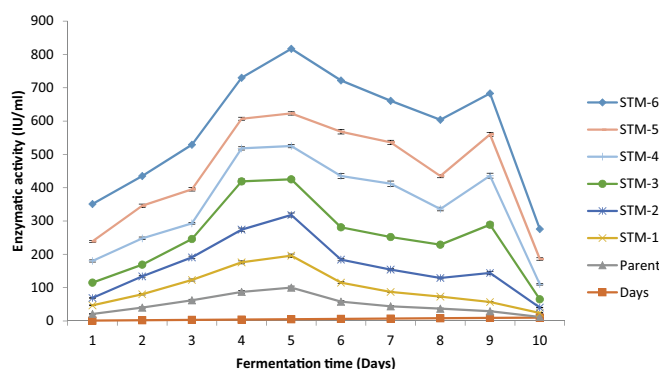


**Fig. 7.** Survival curve of colonies of *Sporotrichum thermophile* at time intervals of EMS treatment.

**Table 8**

Comparison of phytase production between parent and EMS-generated mutant strains.

Strain	Productivity folds	
	(U/l/d)	Increase
Parent	6185 ± 2.43	1
STM-1	15341 ± 2.57	2.5
STM-2	18342 ± 2.19	2.9
STM-3	15432 ± 2.78	2.5
STM-4	16543 ± 2.67	2.6
STM-5	17634 ± 2.64	2.8
STM-6	19875 ± 2.55	3.2



**Fig. 8.** Activity profile of EMS-mutant strains under fermentation conditions.

Due to high production expenditure, deficiency of wanted characters and less yield in the available phytase, the industry is not adopting this (Krishna and Nokes, 2001). So, there is a requirement for identifying the best phytase with best wanted enzymatic characteristics, high yield, and low input. The outcomes of mutagenesis showed an incredible improvement in phytase production rates

which is suggestive of its potential for industrial application. *Sporotrichum thermophile* mutants outperform the phytase production rates in comparison to *Sporotrichum thermophile* with 10,100 U/l (Singh and Satyanarayana, 2006) and recombinant *Escherichia coli* with 2250 U/l. Apart from the characteristics of high production, pH tolerance and temperature stability; it is possible to substitute for other commercially available phytase supplements.

## 5. Conclusion

All the above studies show that the mutants produced through gamma radiations and EMS have a greater potential of phytase enzyme production compare to the parent strain. It is further revealed that the mutant production through EMS has greater the potential of phytase production and enzymatic activity.

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