

Statistical Optimization, Partial Purification, and Characterization of Phytase Produced from *Talaromyces purpureogenus* NSA20 Using Potato Peel Waste and its Application in Dyes De-colorization

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Abstract: In this study, *Talaromyces purpureogenus* NSA20 as phytase-producing marine fungus was isolated and identified morphologically and genetically and deposited in Gene Bank with accession number MW031769.1. One factor at a time (OFAT) optimization was performed, where the result revealed that potato peel waste (1.5%) as a substrate was the highest for phytase production at 6 days, where the maximum activity of phytase was 138.4 U/ml. Moreover, Box–Behnken design as response surface methodology was carried out for statistical optimization of phytase production by *T. purpureogenus* NSA20. Statistical optimization illustrated that the optimized medium for phytase production increased 1.57 fold compared to the OFAT optimized medium. Partial purification of phytase was carried out, where the enzyme after precipitation with ammonium sulfate (80%) was 2.6-fold purified phytase, and the yield was 39.8 %, the specific activity was 31.19 U/mg proteins. Additionally, partially purified phytase was characterized; the maximum activity of phytase at Fe⁺⁺ 0.1% and pH 5.5 at 37 oC was 350 U/ml. Eventually, phytase was applied for crystal violet and methyl red decolorization, where decolorization percentages of crystal violet and methyl red were 85.5% and 75% at 120 min, respectively.

Keywords: *Talaromyces purpureogenus*; phytase; statistical optimization; Box–Behnken design; dye decolorization.

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1. Introduction

Phytic acid [myo-inositol hexakisphosphate (IP6)], known as phytate, organic phosphorous storage, is in the form of phytic acid in most plant tissues [1]. Many crops are useful sources of essential nutrients; however, they contain high phytic-acid values, which may be considered an anti-nutritional factor [2, 3]. Phytic acid is an indigestible constituent for humans and animals; phytic acid has a strong binding affinity to cations such as iron, magnesium, calcium, and zinc, forming complexes insoluble in the intestinal, which leads to inhibition for the absorption of minerals [4, 5]. Moreover, phytic-acid complexes form with protein molecules, causing lower digestibility and solubility, reducing their nutritive value [6, 7]. Besides, phytic-acid chelates vitamin (B3), causing Pellagra, a vitamin- deficiency disease. Phytases (myo-inositol hexakis-phosphate 3-phosphorylase, EC 3.1.3.8 and myo-inositol

hexakis-phosphate 6-phosphorylase, EC 3.1.3.26) are classified as the family of histidinic acid phosphatase which hydrolyzes of phytate to lower phosphorylated myo-inositol derivatives or in some cases to free myo-inositol and inorganic phosphate (Pi) [8]. Phytase is an enzyme that frees the in-organic phosphorous from the phytate complex. It addresses both the eutrophication problem and anti-nutritional; this results in increased availability of amino acids, minerals, trace elements, and phosphate [2]. Phytate degrading enzymes (phytases) have wide applications; it is an enzyme of economic importance due to its potential applications in the food and feed industries, in the formation of myo-inositol phosphate, in agriculture, and the pulp and paper industry [9-12]. But the commercial use is hindered by the high cost of production, which is attributed to the higher cost of the substrate and downstream methods [13, 14]. The high production cost can be overcome by the usage of cheap substrates like residues of agriculture [15]. Some agricultural waste such as wheat bran, citrus peels, wheat straw, rice bran, soybean meal, corn cobs, oil cakes, coconut oil cakes, and corn bran can be utilized as the best carbon source for phytase production. Phytases are widespread, and they are present in many plants, animal tissues, and microorganisms (bacteria, yeasts, and fungi) [16, 17]. Fungi are known for their ability to produce extracellular-enzymes compared to intracellular-enzymes produced by bacteria and yeast cells, and this makes fungi cells attractive for large-scale production of enzymes [18]. Fungi and their metabolites are used in various applications and modern technologies [19-23]. Over 200 fungal cell isolates starting to the genera *Penicillium*, *Mucor*, *Aspergillus*, and *Rhizopus* have been identified as phytases active producers. *Aspergillus* species, namely *Aspergillus ficcum*, *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus fumigatus*, and *Aspergillus carbonarius* are considered as a good producers of phytase enzymes of industrial importance, and the most phytase source is *A. niger* [24]. Statistical experiment design provides an efficient optimization approach. Statistical experiment design is suitable for predicting the interactions between variables and identifying the most significant components in the medium [25]. A combination of factors starting a certain optimum factor response can be identified using factorial design and utilize of surface response methodology [25]. Increase the phytase production can become significant by employing statistical optimization techniques. This is because statistical optimization gives the optimum media with a minimum number of experiments in a short time while also considering the interaction between selected components [26]. This study aimed to isolate and identify a marine fungus that can produce phytase enzymes. Also, to optimize the production of phytase using response surface methodology to obtain the maximum production. Eventually, applying phytase in the dyes decolorization processes.

2. Materials and Methods

2.1. Fungi isolation and culture conditions.

Fungi were isolated from red seawater at Sharm El-Sheikh Governorate. Isolation of fungi was carried out by plating the inoculum on malt extract agar (MEA) (Oxoid) after serial dilutions of sample and incubated at 30 °C for 3-4 days. Contaminations of bacteria were inhibited by chloramphenicol 10µg mL⁻¹ after autoclaving. Colonies morphology were individually picked up and re-inoculated on (MEA) for purification, and the fungal cultures were maintained on (MEA), incubated at 30°C for 7 days, and stored at 4°C [27].

2.2. Morphological and molecular identification of the fungus.

Fungal isolate NSA20 was selected for phytase production. Morphological identification of the fungus was carried out by observing the morphological characteristics (color, texture, and appearance) and microscopic characteristics using a light microscope [28, 29]. Molecular identification was performed based on the 18S rRNA gene. Genomic DNA was extracted utilizing the protocol of DNA purification. The regions were amplified in (PCR) utilizing the genomic DNA as template and primers NS1 5' (GTA GTC ATA TGC TTG TCT C) 3' and NS8 5' (TCC GCA GGT TCA CCT ACG GA) 3'. The 18S rRNA gene was compared against the Gen-Bank database utilizing the NCBI-BLAST program. Sequences were then compared with sequences in the Gen-Bank database utilizing BLASTN. Multiple sequence alignment was done, and a phylogenetic tree was constructed by software program.

2.3. Production of phytase.

T. purpureogenus NSA20 was inoculated in 250ml Erlenmeyer-flask containing 50ml of phytase screening medium containing (g/L): Glucose 10.0; (NH₄)₂SO₄ 3.0; KCl 0.5; MgSO₄.7H₂O 0.5; CaCl₂ 0.1, Calcium phytate 0.5%, pH 5.5) and incubated at 30 °C on a rotary shaker at 150 rpm, after 6 days fermentation, centrifuged at 4,000 rpm for 15 min, 4°C. the filtrate obtained was utilized in the phytase assay.

2.4. Phytase and total protein assay.

Phytase activity was evaluated by measuring the amount value of liberated inorganic phosphate as described by [30]. The reaction mixture consisted of 0.9 ml of acetate buffer (0.2 M, pH 5.5) containing 1 mM phytate and 0.1 ml of enzyme solution. After incubation for 30 mins at 37 °C, the reaction was ended by adding 1 ml of 10% trichloroacetic acid. The liberated inorganic phosphate was measured according to [26]. One unit of phytase is defined as the amount value of enzyme releasing 1 µmol of inorganic phosphorus per ml per minute under the assay conditions. The total protein content was carried out according to Bradford (1976) method.

2.5. Optimization of culture conditions for the production of phytase.

2.5.1. Effect of agricultural wastes on phytase production.

The different agricultural wastes as wheat bran, corn cobs, rice bran, rice straw, corn starch, soybean powder, orange peel, potato peel, and peanut peel were used as substrates for phytase production. All substrates were locally obtained, washed, dried at 70°C, and milled utilizing a grinder before use. 0.5% of the different agricultural wastes were added as a carbon source in fermentation media inoculated with *T. purpureogenus* NSA20. Then, phytase activity was determined. One substrate was further selected to achieve the maximum phytase activity. The best waste concentration for maximum phytase activity was investigated using different concentrations ranging from 1 to 25 mg/ml.

2.5.2. Effect of incubation time on phytase production.

To measure the optimum incubation time for *T. purpureogenus* NSA20 growth and phytase production, the fungal culture was cultivated in production medium and incubated for different incubation times 3, 4, 5, 6, and 7 at 30 °C on an incubator shaker at 150 rpm.

2.5.3. Box–Behnken design.

Based on the preliminary one factor-at-a-time experiments, factors that strongly affect phytase production were selected for Box–Behnken design; potato peel concentration, incubation time, and potassium di-hydrogen phosphate (KH₂PO₄). Table 1 shows selected factors and their levels for Box–Behnken design, three levels for each factor were selected; where levels were (1, 1.5 and 2.0 %), (5, 6, and 7 days) and (0, 0.5, and 1.0 g/l) for potato peel concentration, incubation time and KH₂PO₄ respectively. The model's significance was determined by analysis of variance; the regression equation was obtained, a P value less than 0.05 indicates that the model term is significant. The fit of the model (R²) was studied, and the results were closer to show a better correlation between experimental and predicted values. Moreover, a response optimizer was carried out to detect the optimum conditions for phytase production.

Table 1. Factors and their levels were chosen for Box–Behnken design.

Factors	Name	Levels		
		-1	0	+1
X1	Potato peel conc. (%)	1	1.5	2
X2	Incubation time (Days)	5	6	7
X3	KH ₂ PO ₄ (g/l)	0	0.5	1

2.6. Partial purification of phytase.

The crude phytase enzyme was partially purified by precipitation of ammonium sulfate to 80% saturation. The precipitated phytase was collected using centrifugation at 10,000 rpm for 10 min at 4°C and dissolved in a minimum volume of acetate buffer (0.2M; pH 5.5). The solution of the enzyme was dialyzed at 4 °C against the buffer for 24 h. The dialysis was concentrated through a freeze dryer and dissolved in a minimum volume of the buffer. Then, phytase activity was estimated.

2.7. Characterization of partially purified phytase.

The enzyme precipitated by ammonium sulfate and desalted by dialysis was used to characterize partially purified phytase.

2.7.1. Effect of different pH values.

The optimum pH value was determined by measuring the number of partially purified phytase activities between pH 4.0 and 7.0 using acetate buffer 0.2M (pH 4 -5.5) and phosphate buffer 0.2M (pH 6-7).

2.7.2. Effect of different temperatures.

For determining the optimum temperature for the activity of partially purified phytase, the enzyme assays were carried out in the temperature range between 30 and 60°C.

2.7.3. Effect of metal ions and surfactants.

The influence of metal ions and surfactants on partially purified phytase activities was studied. Metal ions such as KCl, CaCl₂, MgSO₄.7H₂O, MnCl₂, ZnSO₄, CuSO₄, FeSO₄, and surfactants, i.e., Triton 100, Tween-20, and Tween-80, were added to the mixture at (0.1% w/v) to determine their effect on phytase activities. Then, enzyme activities were assayed.

2.8. Application of phytase in dyes decolorization processes.

The partially purified phytase was applied to de-colorize two synthetic dyes (Crystal violet and Methyl red). The reaction consisted of 4 mL of each dye dissolved in distilled water and 1 mL of purified phytase in a total volume of 5 mL. A decrease in the maximum absorbance of dyes (Crystal violet, 575 nm, and Methyl red, 520 nm) was determined every 30 min after incubation of reaction mixtures at 37 °C. At the parallel, control without phytase was processed to confirm non-enzyme decolorization. All decolorization experiments were tested in triplicate.

2.9. Statistical analysis.

Means of three replicates and standard errors were calculated for all obtained results, and the data were subjected to analysis of variance means using sigma plot 12.5 programs.

3. Results and Discussion

3.1. Identifications of fungal strain.

Colonies of fungal isolate NSA20 are moderate growing and a dark grey-green, sporulation moderately dense, conidiophores biverticillate, subterminal branches absent; stipes smooth-walled, phialides acerose, 3–5 per metula, conidia smooth, ellipsoidal Figure 1A. The 18S rRNA gene analysis of the fungal isolate NSA20 showed similarity (98.56 %) with *Talaromyces purpureogenus*, as shown in a phylogenetic tree Figure 1B. The Gen Bank accession number for strain NSA20 nucleotide sequence is MW031769.1, identified as *Talaromyces purpureogenus* NSA20.

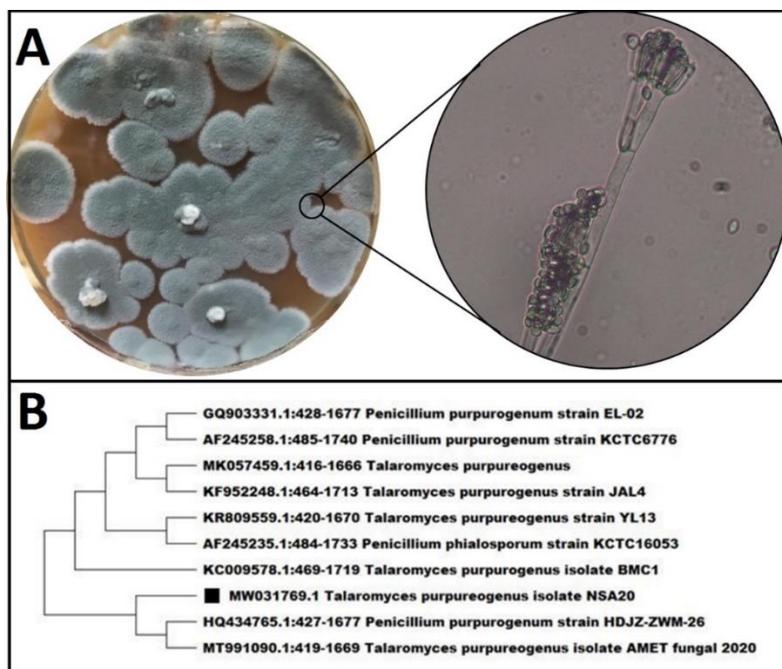


Figure 1. Identification of fungal strain NSA20 (A-B): A) Morphological identification. B) Neighbor-Joining phylogenetic tree of 18 s rRNA gene.

3.2. Phytase production by *T. purpureogenus* NSA20.

The results showed that *T. purpureogenus* NSA20 was the highest for phytase production in fermentation medium (103.5 U/ml). [31] showed that *T. lanuginosus* strain

produced the highest value of phytase (25 U/L). [32] reported that *A. niger* gave the highest level of phytase activity, 138.6 U/mL. [30] indicated that *P. purpureogenum* produced a high amount of phytase (150 U/ml). Also, [33] mentioned that *A. heteromorphus* produced the highest value of phytase activity 14.80 U/ml. Likewise, [34] indicated that phytase enzyme had been produced from *Aspergillus oryzae*.

3.3. Effect of agricultural wastes on the production of phytase.

The utilization of agricultural waste products is considered the main goal of many researchers to get cheap mediums and solve the problems that resulted from the accumulation of these wastes. Within this context, different agricultural wastes as wheat bran, corn cobs, rice bran, rice straw, corn starch, soybean powder, potato peel, orange peel, and peanut peel were used as a substrate for the production of phytase utilizing *T. purpureogenus* NSA20. Figure 2A showed that all the agriculture waste used as substrates had phytase activities despite the variety in the activity value. The highest phytase activity (114.5 U/ml) by *T. purpureogenus* NSA20 was observed when potato peel was utilized as a substrate, followed by peanut peel and orange peel produce (99.25, 97.7 U/ml), respectively. While soybean powder, wheat bran, and rice bran were produced (96.18, 95.9, and 91.14U/ml), the lowest productivity of phytase was noticed with corn cobs waste with a yield of 82.28 U/ml. The results proved that the potato peel waste was a good substrate for phytase production by *T. purpureogenus* NSA20. The selection of substrate for the formation of an enzyme is a key factor and significant role in producing suitable metabolites [35]. Other results were shown by [36, 37] indicated that the phytase formation by *B. cereus*, *P. stewartii*, and *Mucor jalaludini* was increased using the rice bran supplementation. Awad *et al.* indicated that the highest yield of the enzyme by *P. purpureogenum* GE1 fungus grown on corn cob was 46 ± 2.8 U/g ds [30]. Others reported that the highest phytase production by *R. oryzae* fungus grown on coconut oil was 30.1U/gds [38]. While others found that *A. ficuum*NRRL3135 had the highest productivity (15 IU phytase activity/g dry matter) on wheat bran [39]. Others reported that the phytase production by *M. racemosus* utilizing combinations of numerous oil cakes with wheat bran which gave the highest productivity of phytase 32.2 U/gds [40]. [41] studied the application of *A. niger*, *R. oryzae*, and *Neurospora sitophila*, on mixed soybean curd and rice straw. *Neurospora sitophila* showed the highest productivity of phytase at 195.66 U/g. [31] reported that *Thermomyces lanuginosus* gave a high value of phytase (25U/L) on medium containing rice flour as the main carbon source. Also, rice flour was a good carbon source for phytase formation using *A. niger* [42].

3.4. Effect of potato peel concentrations on phytase production.

The best substrate waste for the production of phytase was potato peel utilizing *T. purpureogenus* NSA20 0. The influence of potato peel concentrations on phytase production using *T. purpureogenus* NSA20 was investigated. Figure 2B illustrates the high-value production of phytase (120.3 U/ml) with a waste concentration at 1.5 %w/v. Further decrease or increase in the potato peels concentration leads to decrease phytase production. Other results were reported by [31], who studied the influence of rice flour concentrations on phytase production using *T. lanuginosus* and showed the optimal rice flour concentration for maximum activity of phytase was 5% (w/v) at 200rpm after the 4th day of fermentation.

3.5. Effect of incubation time on phytase production.

The influence of incubation time on the production of phytase by *T. purpureogenus* NSA20 was studied. Figure 2C shows high phytase production (138.4 U/ml) was observed on the 6th day of incubation. After that, the phytase activities decreased gradually as incubation time increased due to reduced nutrient level of medium or autolysis of the mycelium occurred [43]. Singh and Satyanarayana showed that the increase in phytase production was on the fifth day of incubation by *Sporotrichum thermophile* [44]. Researchers have shown that the maximum production of phytase from *A. niger* was evaluated on the 4th day (38.5U/ml in SSF and 25.6 U/ml in SMF) [45]. A previous study reported that the highest value of phytase production by *A. tubingensis* was after 4 days using wheat bran [46]. Lata *et al.* mentioned that the phytase activity was initially detected at 48 hr and increased with time [33]. A high growth level and the production of phytase (17.88 U/ml) were observed at 120 hrs of incubation time by *A. heteromorphus*. However, high phytase activity (68 U/ml) was shown on the 11th day of incubation by *A. niger* [47].

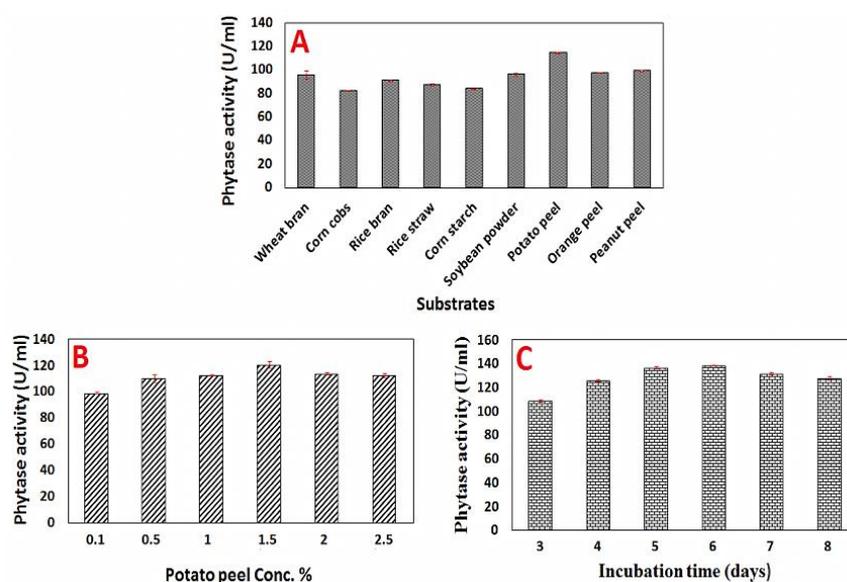


Figure 2. Influence of agricultural wastes (A), the concentration of Potato peel (B) and, incubation time (C) on phytase production by *T. purpureogenus* NSA20.

3.6. Response surface methodology (Box–Behnken design).

Box–Behnken design was performed to make more interactions between factors and their levels for getting the target product. Box–Behnken design is more efficient and robust than other designs as full multi-level design, central-composite design (CCD), and doehlert-design, despite its poor coverage of the angle of the nonlinear design space [48]. Table 2 illustrates Box–Behnken design which includes three factors (potato peel concentration, incubation time, and KH_2PO_4) with three-level for each. Results showed high phytase activity in run no. 14 with potato peel 1.5%, incubation time 7 days, and KH_2PO_4 1.0 g/l. The F value (Fisher’s statistical analysis) and P-value (>0.0001) were used for determining the significance of the model. Low P values indicate the high significance of the corresponding coefficient, whereas large t and F values indicate the significance of corresponding coefficients [49]. ANOVA results showed that the model is highly significant where P value < 0.05 . Moreover, model terms KH_2PO_4 , $\text{KH}_2\text{PO}_4 * \text{KH}_2\text{PO}_4$, and Time* KH_2PO_4 were significant for phytase production, as shown in

Table 3. The correlation coefficient (R²) measures how much the observed response variability can be explained by the experimental parameters and their interactions [50]. R² of the model is 0.991, which indicates that 99.10% of the variability in the response could be expressed by the model. The production of phytase activity (U/ml) was predicted by the following model equation:

$$\text{Phytase activity} = -387 + 168.2 X_1 + 119.4 X_2 + 220.9 X_3 - 36.0 (X_1 * X_1) - 9.32 (X_2 * X_2) - 223.5 (X_3 * X_3) - 8.25 (X_1 * X_2) - 13.3 (X_1 * X_3) + 21.80 (X_2 * X_3).$$

Table 4 shows KH₂PO₄ only factor had a significant effect on phytase activity. While as potato peel and incubation time factors had no significant effect on phytase production. Moreover, all interactions had no significant effect except potato peel and KH₂PO₄, as shown in Figure 3. The optimal levels of the three components as obtained from the maximum point of the polynomial model were estimated using response optimizer in Mini tab 17 software and found to be: potato peel 1.42 g/l, incubation time 6.67 d (160.08 h), and KH₂PO₄ 0.778 g/l with a predicted phytase activity of 217.36 U/l. Finally, this statistical analysis revealed that the optimized medium for phytase production increased to 1.57 fold compared to one factor at a time (OFAT) optimized medium.

Table 2. Effect of different parameters on phytase production using Box–Behnken design.

Run	Potato peel concentration (%)	Incubation time (Days)	KH ₂ PO ₄ (g/l)	Phytase activity (U/ml)		
				Actual	Predicted	Residual
1	1.0	5	0.5	168	169.881	-1.88125
2	2.0	5	0.5	180.8	182.244	-1.44375
3	2.0	6	0.0	92.83	86.885	5.94500
4	2.0	7	0.5	188	186.119	1.88125
5	1.5	6	0.5	200.2	200.433	-0.233333
6	1.0	6	0.0	82.48	76.0975	6.38250
7	1.5	5	0.0	81.5	86.0012	-4.50125
8	1.0	6	1.0	185	190.945	-5.94500
9	1.5	6	0.5	200.9	200.433	0.466667
10	1.5	5	1.0	180.2	172.374	7.82625
11	2.0	6	1.0	182	188.382	-6.38250
12	1.0	7	0.5	191.7	190.256	1.44375
13	1.5	6	0.5	200.2	200.433	-0.233333
14	1.5	7	1.0	210.8	206.299	4.50125
15	1.5	7	0.0	68.5	76.3262	-7.82625

Table 3. Analysis of variance for Box–Behnken design.

Source	Degree of freedom	Sum of squares	Mean of squares	F-value	P-value
Model	9	36005.5	4000.6	61.22	0.000
X ₁	1	33.8	33.8	0.52	0.504
X ₂	1	294.0	294.0	4.50	0.087
X ₃	1	23402.6	23402.6	358.11	0.000
X ₁ ²	1	298.4	298.4	4.57	0.086
X ₂ ²	1	320.6	320.6	4.91	0.078
X ₃ ²	1	11523.5	11523.5	176.33	0.000
X ₁ X ₂	1	68.1	68.1	1.04	0.354
X ₁ X ₃	1	44.6	44.6	0.68	0.447
X ₂ X ₃	1	475.2	475.2	7.27	0.043
Lack-of-Fit	3	326.4	108.8	666.18	0.001
Pure Error	2	0.3	0.3		

A value of P < 0.05 indicates that the model term is significant. R²= 0.9910% Adj R²= 0.9748

Table 4. Regression analysis of a full second-order polynomial model for optimization of phytase production.

Term	Coefficient	Standard error of coefficient	T-Value	P-Value
Intercept	200.43	4.67	42.94	0.000
X ₁	2.06	2.86	0.72	0.504
X ₂	6.06	2.86	2.12	0.087
X ₃	54.09	2.86	18.92	0.000
X ₁ ²	-8.99	4.21	-2.14	0.086

Term	Coefficient	Standard error of coefficient	T-Value	P-Value
X_2^2	-9.32	4.21	-2.21	0.078
X_3^2	-55.87	4.21	-13.28	0.000
$X_1 X_2$	-4.13	4.04	-1.02	0.354
$X_1 X_3$	-3.34	4.04	-.083	0.447
$X_1 X_3$	10.9	4.04	2.70	0.043

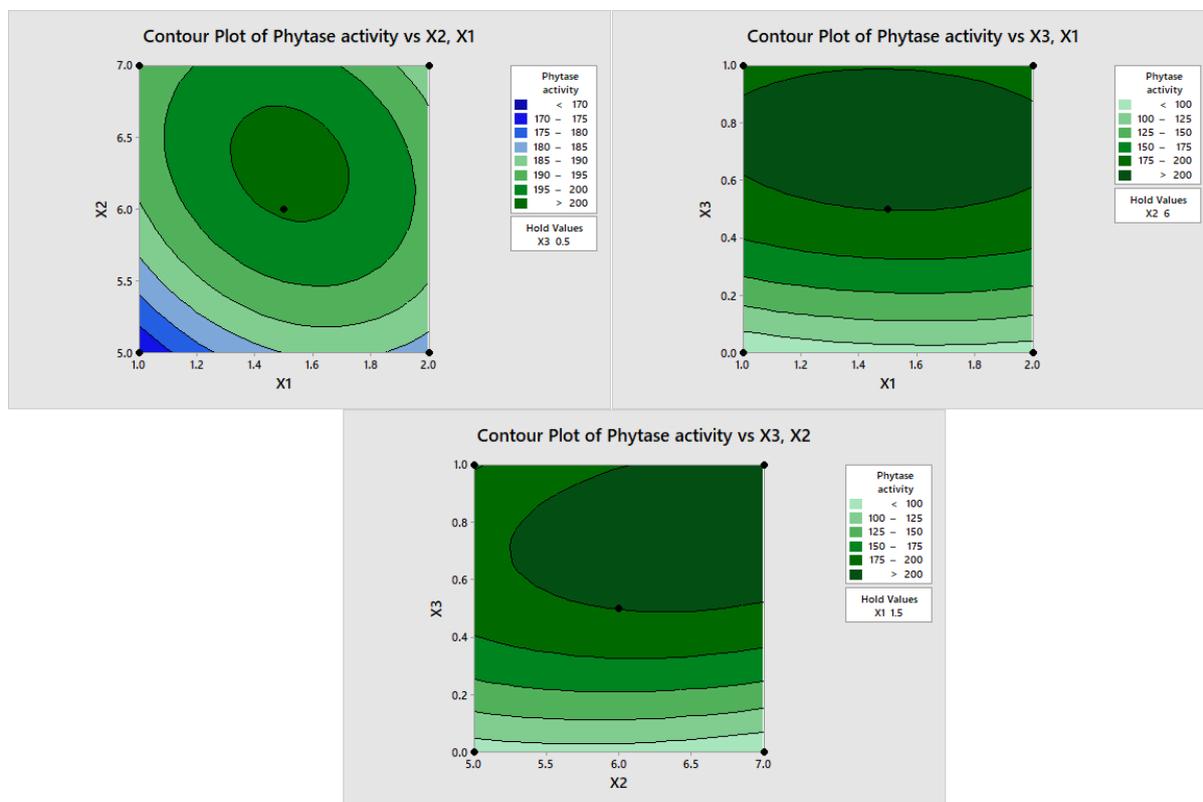


Figure 3. Contour plot of phytase activity vs. interactions between potato peel (%), incubation time (d), and KH_2PO_4 (g/l).

3.7. Partial purification of phytase.

The crude enzyme produced from *T. purpureogenus* NSA20 using potato peel waste 1.5% was partially purified by ammonium sulfate (80%) precipitation and dialysis. The results summarized in Table 5 recorded that the enzyme after precipitation with ammonium sulfate(80%) was 2.6-fold purified phytase, and the yield was 39.8 %, the specific activity was 31.19 U/mg protein. Ajith *et al.* studied that the phytase's purification from *A. foetidus* using ammonium sulfate followed by chromatography separation, demonstrates that phytase was purified about 23.4-fold with the recovery of 13% from the crude enzyme [51]. In a previous study reported that the phytase from *T. lanuginosus* was purified about 9.1-fold with a production of 5.1 % [31]. The phytase enzyme produced from *Thermomyces lanuginosus* was purified, resulting in 3.44% production and 40.75 fold [52]. The higher purification fold may be due to the beginning protein synthesized on wheat bran through SSF. The ratio of phytase is lower in the crude protein, which leads lower fold.

Table 5. Partial Purification of phytase produced by *T. purpureogenus* NSA20.

Purification steps	Total activity (U/ml)	Total protein (mg)	Specific activity (U/mg)	% Yield	Fold
Crude enzyme	5270	432.5	12.18	100	1
Precipitation with ammonium sulfate (80%)	2096	67.2	31.19	39.8	2.6
After dialysis	627.5	8.75	71.7	11.9	5.9

3.8. Characterization of partially purified phytase.

3.8.1. Effect of different pH values.

Effect of pH on phytase activity produced from *T. purpureogenus* NSA20 at pH range from (4-7) was observed in Figure 4A. The maximum activity of phytase at pH 5.5, other results were reported that the high activities of phytase at pH 3.5, 5.4, and 5.5 [51, 53]. The optimum pH was 5.5 in the case of phytase originated from thermophilic-fungi *A. fumigatus*, *M. thermophile*, and *S. thermophile* [54]. The pH optimum at phytase from *Thermomyces lanuginosus* and *T. aurantiacus* was at pH 5.0 [42, 55].

3.8.2. Effect of different temperatures.

The influence of temperatures on phytase activity produced from *T. purpureogenus* NSA20 was studied in the range from 30 to 70 °C. Figure 4B reported that the highest value of the activity of phytase was observed at 37 °C. Another result was reported by [51], who studied the influence of temperature on phytase activity from *A. foetidus* and appeared activities in the range 4 - 80 °C with high activity at 37 °C. A previous study mentioned that the optimum temperature of phytase produced from *T. lanuginosus* was measured at 70°C [26]. The phytase produced from *A. niger* appeared a high value of specific activities at temperatures ranged from 52–55°C [56].

3.8.3. Effect of metal ions and detergents.

The effects of metal ions and detergents on phytase activities were investigated by adding various salts and detergents to the reaction mixture at (0.1% w/v). The results recorded in Figure 4C reported that ion Fe^{++} 0.1% increased the activities of phytase with 6%,

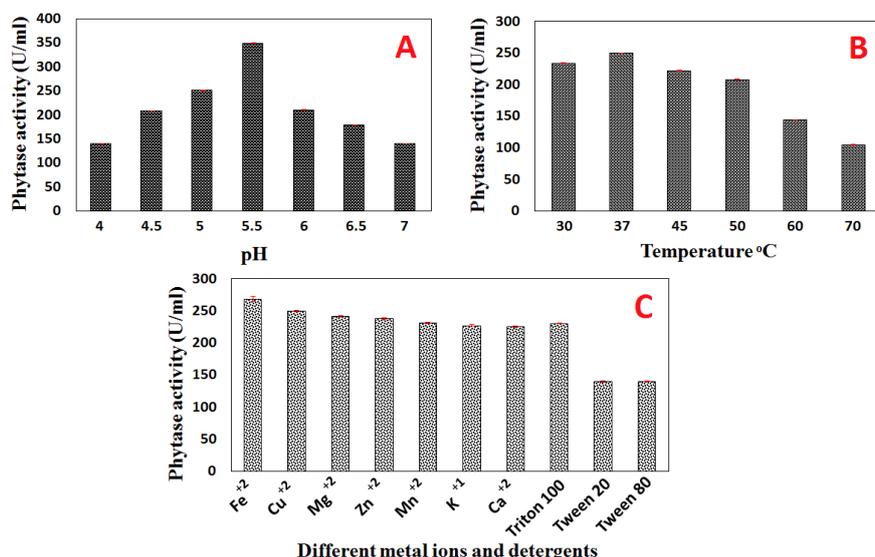


Figure 4. Influence of pH (A), temperature (B), and metal ions and detergents (C) on phytase activity from *T. purpureogenus* NSA20.

However the metal ions Ca^{++} , K^{+} , Mg^{++} , Mn^{++} , Cu^{++} and Zn^{++} not effect on activities. The phytase was more resistant to inactivation by metal ions: The detergents Triton -100 at 0.1% showed no effect on phytase activity, while Tween-20 and Tween-80 at 1% showed inhibitory effects on the phytase activity (44.3%). [31] reported that the presence of 1 mM

Fe^{3+} , Fe^{2+} , and 5 mM Ca^{2+} , Mg^{2+} and K^{+} , ions resulted in 13-22% increase yield of activity, whereas the presence of 5 mM of Ag^{+} , Co^{2+} and Zn^{2+} , ions resulted from an inhibitory effect on phytase activity of *T. lanuginosus*. Na^{+} ion does not affect on phytase activity of *T. lanuginosus*. Gulati *et al.* reported that Cu^{2+} ion has an inhibitory effect on phytase produced from *T. lanuginosus* strain, but each of Ca^{2+} , Mg^{2+} , Fe^{2+} , K^{+} , Mn^{2+} , Na^{+} , Ba^{+} , Zn^{2+} ions decrease the phytase activity in a concentration of 5 mM [52]. Cu^{2+} ions at 1mM inhibited phytase produced from *Neurospora crassa*, and Ca^{2+} , Mg^{2+} , Fe^{2+} , Co^{2+} , Mn^{2+} , Zn^{2+} ions have no significant effect on phytase activity [57]. Others reported that the Fe^{2+} and Fe^{3+} ions have an inhibitory effect at 1mM on phytase formation by *E. nidulans*, *A. terreus*, *A. niger*, and *A. fumigatus* [58].

3.9. Dye decolorization by phytase.

The partially purified phytase from optimized was applied to de-colorize two synthetic dyes; Crystal violet and Methyl red. The decolorization percentage of two dyes was increased by increasing gradually in time and appeared its high effect after 120min Figure 5. The results showed that decolorization percentages of the Crystal violet and Methyl red dyes by partially purified phytase were 85.5% and 75%, respectively, after 120 min incubation, as shown in Figure 5. Aghaie-Khouzani *et al.* optimized and purified enzyme from the culture of *P. variable* and evaluated its utilized in decolorization of Sudan black, Amido Black, Crystal violet, Rimazol brilliant blue, and Bromothymol blue; the data appeared that the decolorization of five dyes were 84, 54, 94, 93 and 87% respectively, after 180 min [59]. Another study utilized the fungal cells of *Aspergillus niger* to decolorization of azo dyes (Reactive red (4BL) and Reactive yellow (4GL), the decolorization percentage was 92.42 % and 98.62 %, respectively on shaker after 7 days [60].

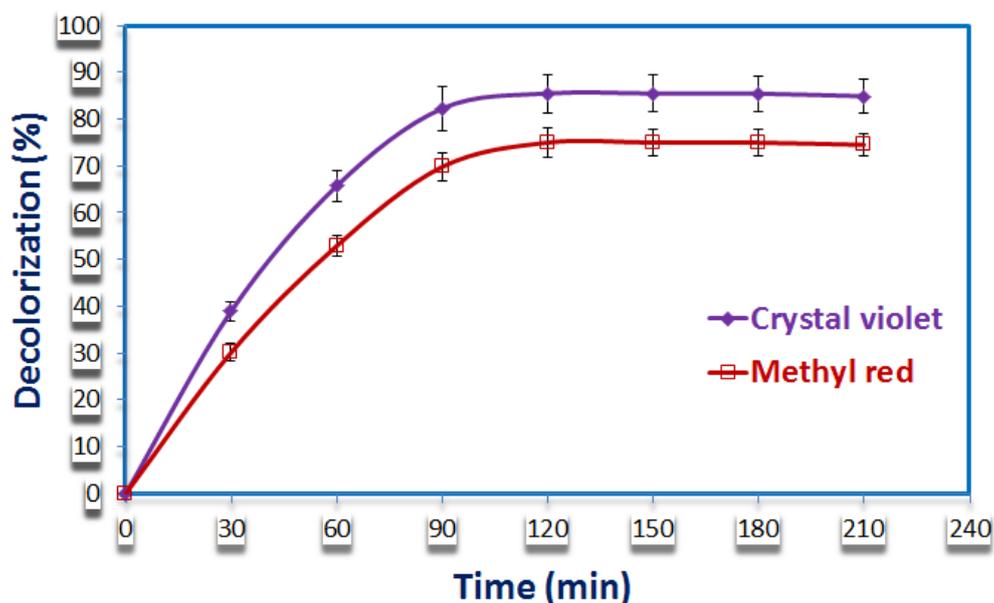


Figure 5. Dye decolorization using partially purified phytase from *T. purpureogenus* NSA20.

4. Conclusions

A promising *T. purpureogenus* NSA20 as a phytase-producing marine fungal strain was isolated and identified genetically in the current study. Results revealed that potato peel waste as a substrate for *T. purpureogenus* NSA20 is the highest for phytase production among other

wastes. Statistical optimization using Box–Behnken design as response surface methodology was carried out, where statistical analysis illustrated that optimized medium for phytase production increased to 1.57 fold compared to OFAT optimized medium. Moreover, partial purification of crude enzyme and characterization of partially purified phytase was performed. Finally, partially purified phytase was effective for decolorizing crystal violet and methyl red quickly.

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Conflicts of Interest

The authors declare no conflict of interest.

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