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Isolation of Phytase Producing Fungi and Optimization of Production Parameters

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ABSTRACT

Seventeen cultures of phytase producing fungi were isolated from different leguminous soil samples. Among these PF-3 exhibited maximum phytase activity. Studies were carried out to optimize the physical and chemical parameters. The optimum productivity of phytase was achieved with optimized process parameter such as Calcium phytate (0.5%) as carbon source, $(\text{NH}_4)_2 \text{SO}_4$ (0.1%) as nitrogen source, agitation speeds at 160 rpm, Tween-80 at (0.5% v/v) as a surfactant, incubation temperature of 30°C , initial pH of 6, incubation period of 4 days, 5% inoculum level. The maximum production of phytase was under the optimized conditions an increase the yield of 1.63 times.

Keywords: Isolation, phytase, optimization, fungi, enzyme activity.

1. INTRODUCTION

Phytase is an enzyme that hydrolyses phytic acid to myo-inositol and phosphoric acid and classified as histidine acid phosphatases. Phytic acid is chemically myo-inositol 1, 2, 3,4,5,6 – hexakisdihydrogen phosphate -IP6. It is the major storage form of phosphate in plants. Most foods of plant origin contain 50-80% of their total phosphorous as phytate. Some of the commercially important phytase producing fungi are: *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*.

2. MATERIALS AND METHODS

All the soil samples were collected near the root of leguminous plants at 4-6 inches depth in sterile screw capped test tubes.

Antibacterial agent (Penicillin –5 units/ml) was incorporated to control the bacterial contamination. Medium poured into 6 inches sterile petri plates and incubated at 28°C for 3days. Each colony producing a translucent zone was considered a potential phytase producer. The diameter of the translucent hydrolyzed zone is an indication the amount of phytase produced. Around 17 isolates were screened and selected according to their best-hydrolyzed zone diameters. The isolates were inoculated on Potato Dextrose Agar (PDA) medium slats and maintained as master cultures.

- Effect of various Incubation Temperatures (25°C , 30°C , 37°C , 50°C for 6 days)
- Effect of pH (5-9)
- Effect of various Incubation Periods (30°C for 8 days)
- Effect of Various Surfactants(Tween-80 and Triton X-100)

Czepak's medium without phosphate

NaNO ₂	-	2 g
MgSO ₄ .7H ₂ O	-	0.5 g
KCl	-	0.5 g
FeSO ₄	-	0.5 g
Calcium phytate	-	0.5 g
Distilled water	-	up to 1000 ml
pH	-	5-5.6

Table No. 1. Composition of different media for Phytase production

	Quantities (g/L) of different media		
	Medium No. I	Medium No.II	Medium No.III
Corn starch	-	80	-
Glucose	10	30	-
Malt extract	3	-	-
Yeast extract	3	-	2
Sucrose	-	-	10
Peptone	0.5	-	-
Tryptone	-	-	3
MgSO ₄ .7H ₂ O	-	0.5	0.5
MnSO ₄ . 7H ₂ O	-	0.01	0.01
FeSO ₄ . 7H ₂ O	-	0.1	0.01
(NH ₄) ₂ SO ₄	-	-	2
K ₂ HPO ₄	-	0.2	-
KCl	-	-	0.5
pH	6.8	5.4	7.4

Of the three media studied Medium III gave maximum phytase yield - 47U/ml (Fig.2) was selected as a basal medium for subsequent studies.

3. RESULTS AND DISCUSSION

About 17 fungal isolates were isolated from screening procedure. Among isolates, 5 isolates of PF-3, PF-2, PF-1, PF-5, and PF-4 showed significant Phytase activity. PF- 3 was found to be the best phytase producer according to its maximum hydrolyzed zone diameter (15.7mm). Of the three media studied Medium III gave maximum phytase yield - 47U/ml was selected as a basal medium for subsequent studies.

The effect of incubation temperature was studied on phytase production and 30⁰C (48.5U/ml) was found to be optimum temperature. It was found that the maximum phytase production (50 U/ml) was obtained at pH 6. The highest phytase production (52 U/ml) was obtained at 5 % v/v. So the inoculum level of 5% v/v was found to be optimum for maximum phytase production.

Among different carbon sources studied, the highest yield of phytase was obtained with Calcium phytate (64.5U/ml).

Among various both organic and inorganic nitrogen sources better phytase production (73 U/ml) exhibited by (NH₄)₂ SO₄ with optimum concentration of 0.1%. Among various agitation speeds better phytase production (75 U/ml) exhibited at 160 rpm. High phytase production (77 U/ml) exhibited by Tween-80 at concentration of 0.5%v/v. The maximum production of phytase was under the optimized conditions 77U/ml.This represents an increase the yield of 1.63 times.

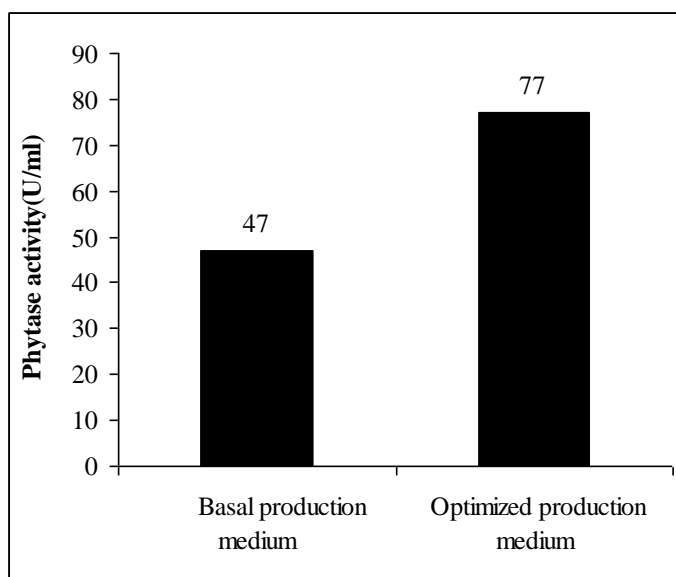


Figure 1: Phytase production in the basal production medium and optimized production medium

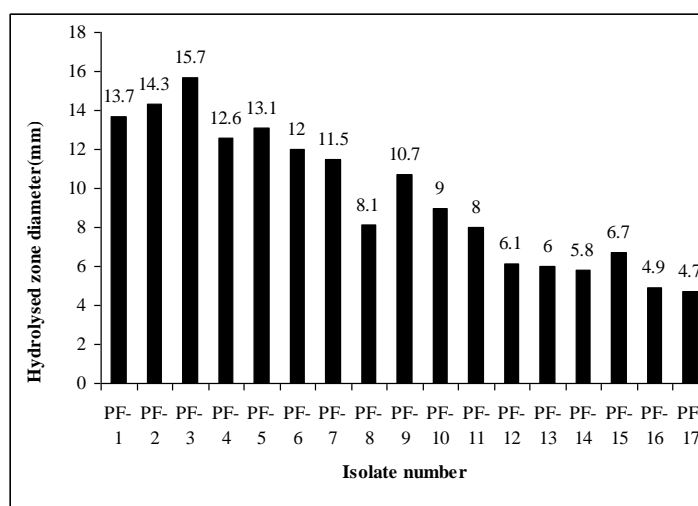


Figure 2: Hydrolysed zones to different fungal screened isolates

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