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Optimization of Phytase Production by *Pseudomonas sp.* Isolated from Poultry Faces

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ABSTRACT

Phytase production in *Pseudomonas* sp isolated from poultry faces was investigated and optimized. in the present investigation. Effect of different agricultural substrates on phytase production by the *Pseudomomas sp* revealed that the maximum amount of phytase was produced with ragi bran as a substrate than other substrates used in the study. All the kinds substrates used in the study for phytase production were observed at 72 hours of fermentation and pH 5 and 37 ºC were observed as the optimum pH and temperature for maximum phytase production at 72 h. Ammonium sulphate and sucrose were observed as the best nitrogen and carbon sources for higher rate phytase production. Similarly Tricalcium phosphate was identified as suitable phosphate source for maximum phytase production by the *Pseudomonas sp.*.

Keywords: Poulty faces, Pseudomonas sp, agricultural substrates, effect of pH, temperature, carbon, nitrogen and phosphate sources – optimization of phytase production.

1. INTRODUCTION

Phytase *myo*-inositol hexaphosphate phosphohydrolase is an enzyme that hydrolyses phytin which comprises 50% to 80% of the total phosphorus in most foods of plant origin. It is a type of anti-nutritional factor commonly present in edible legumes, cereals, and seeds [1]. While preparing animal feeds using these plant materials, phytic acid make unavailability of essential nutrients in the feed hence, phytases have been mainly, used as animal feed supplement in diets mainly for swine and poultry and also for fish. The first commercial phytase products were launched into the market in 1991, and now the market volume is increased to 150 million euro dolar [2].

Phytases have a wide distribution in plants, microorganisms, and in some animal tissues. Several strains of bacteria, yeasts and fungi have been used for production of phytase in large scale for commercial purpose. Many bacterial strain have been studied for the phytase production such as *Pseudomonas* sp., *Bacillus* sp., *Raoultella* sp., *Escherichia coli*, *Citrobacter braakii*, and *Enterobacter*, and including anaerobic rumen bacterial species like *Selenomonas ruminantium*, *Megasphaera elsdenii*, *Prevotella* sp., *Mitsuokella multiacidus*, and *Mitsuokella jalaludinii* [3].

Phytases are mainly applied to reduce phytate content in feed and food stuffs, they are mainly used in bread making; corn wet milling and for production of plant proteins [3]. Supplementing phytases in the feed have been significantly improving phosphorus utilization from phytate to commercially important animals under various dietary conditions. Phytase production is greatly affected by medium composition used for bacterial culture and the phytase production is mainly induced by nutrient and physical conditions. Hence, the present study was carried out to optimize the phytase production by *Pseudomonas sp.* using different nutritional and physical conditions.

2. MATERIAL AND METHODS

2.1. Microorganism and phytase production

The microorganism used in this study was isolated from the poultry faeces from Pillyarpuram poultry farm. The bacterial isolate was identified as *Pseudomonas* sp. by its morphological, physiological and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology. To begin the phytase production, overnight seed culture was prepared by cultivating the strain in nutrient agar, afterwards, 10% of the seed culture was inoculated into 50 ml of production medium containing K_2HPO_4 (0.1%,) NaCl (1%,)$MgSO_4 \cdot 7H_2O$ (0.01%) and peptone (0.5%.) using fermentor. Fermentation was carried out at 32°C for 72 h with 150 rpm. All the experiments were done in triplicate and average values were recorded. After 72 hours of incubation the cells were harvested by centrifugation at 10,000 rpm for 15 minutes and the supernatant was used as a enzyme source for estimation of phytase activity.

2.2. Assay of Phytase production.

Phytase production was analysed using the method suggested by [4]. To the 0.2 ml of enzyme sample, 2.4 ml phytic acid solution (0.32gm Sodium phytate, dissolved in 50ml of 0.2 m sodium acetatic acid buffer with pH 5.5) was added. To this mixture 1ml of 0.1 molar $Mg\ SO_4.6\ H_2O$ and 0.4ml of distilled water were added. The content was incubated at room temperature for 15 minutes and the reaction arrested with the addition of 0.5ml Trichloro acetic acid (10%) and after that 1 ml of distilled water and 2.5ml of Taussky-schoor reagent (freshly prepared) were to

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this mixture, the absorbance was measured at 660nm using spectrophotometer. Phytase activity was calculated using phosphorus standard; 1U phytase activity is equivalent to 1 µg phosphorus released under assay conditions.

2.3. Parameters controlling phytase production

2.3.1. Effect of different agricultural substrates

Phytase production with agricultural substrates was studied by using different substrates such as rice bran, ragi bran, green gram, wheat bran and corn bran at 1% level. This was studied with different time intervals such as 24, 48, 72 and 96 hours..

2.3.2. Effect of different initial pH on phytase production

The effect of incubation pH on phytase production was determined by varying the pH values such as 5, 7 and 9, their influence on phytase production was determined at different time intervals (24, 48, and 72 hours).

2.3.3. Effect of different incubation temperature on phytase production

The organisms inoculated in the production media were incubated at different temperatures such as 27^{o}C, 37^{o}C and 47^{o}C and their influence was noticed at different time intervals (24, 48, 72 and 96 hours).

2.3.4. Effect of different nitrogen sources on phytase production.

The effect of nitrogen sources on phytase production was determined using different organic (malt-extract, yeast extract) and inorganic nitrogen sources (ammonium nitrate, ammonium sulphate (0.5%)) and their influence of nitrogen sources was observed at different time intervals (24, 48, 72 and 96 hours).

2.3.5. Effect of different carbon source

Suitability of different carbon sources such as glucose, lactose, maltose and sucrose were studied at 0.5% level. The influence of carbon sources on phytase production was noticed at different time intervals (24, 48, 72 and 96 hours).

2.3.6. Effect of phosphate on phytase production

The effect of phosphates on phytase production was also studied using Tricalcium phosphate and sodium dihydrogen ortho phosphate at 0.1% level at different time intervals such as 24, 48, 72 and 96 hours.

3. RESULTS AND DISCUSSION

The effect of different agricultural substrates on phytase productions at different time intervals were represented in Table-1 & Fig.1. The results indicated that among the tested substrates ragi bran showed maximum phytase productionwhen compared to other substrates used in the study.

Table-1. Effect of different agricultural substrates on phytase production at different time intervals

Agricultural substrates	Time Intervals			
	24	48	72	96
	Phytase Activity (U/ml)			
Ragi Bran	0.669	0.88	0.891	0.81
Rice Bran	0.105	0.61	0.551	0.598
Green gram	0.575	0.633	0.468	0.399
Corn bran	0.434	0.234	0.639	0.493
Wheat bran	0.305	0.223	0.61	0.315

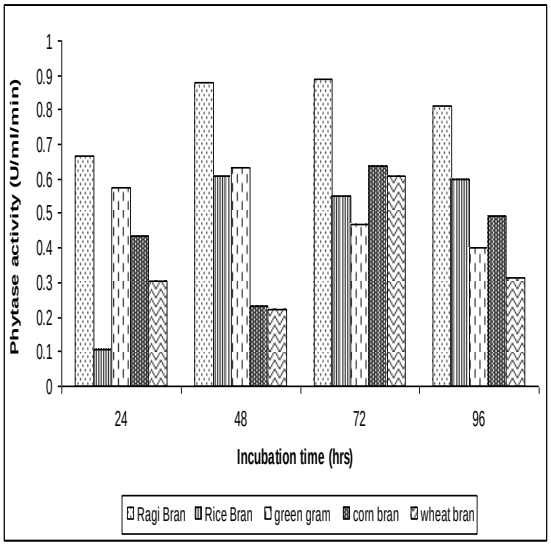

Fig.1. Effect of different agricultural substrates on phytase production at different time intervals

Among the tested different time intervals it was observed that phtyase production was maximum in 72 hours of fermentation. Next to ragi bran, green gram and corn bran showed good results with *Pseudomonas* sp. Production of phytase using agricultural wastes provides many advantages especially reduce the production cost. Many authors have reported the compatible use of agricultural wastes for phytase production by different bacterial strains. It is eported that the phytase production using sesame oil cake by *Sporotrichum thermophile* [5]. Similarly, high level of phytase production was observed with the wheat bran and oil cakes in *Mucor racemosus* [6]. Likewise the level of phytase production in soybean meal by *Aspergillus oryzae* AK9 [7] these results were corroborates with the present investigation.

Effect of pH on phytase production with different time intervals resulted that pH 5 and 72 hours of incubation period were suitable for maximum phytase production (Table.2 & Fig. 2).

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Table-2. Effect of pH on phytase production with different time intervals

pH	Time Intervals			
	24	48	72	96
	Phytase Activity (U/ml)			
5	0.434	0.516	0.657	0.399
7	0.399	0.41	0.633	0.575
9	0.399	0.14	0.61	0.223

This incubation period was observed in all set of experiments with different substrates. These results showed that the phytase produced by this strain was with acidic enzyme synthesized by the organism. Supporting the present study *Saccharomyces cerevisiae* CY are reported to produced phytase in acidic pH and has an optimum pH of 5.5 [8]. Similarly, it was also reported that, phytate degradation by *S. cerevisiae* YS18 in the cultivation medium was high at initial pH 6.0 [9]. Likewise, the maximum phytase production reported in *Mucor indicus* MTCC 6333 at pH 5.0 [10].

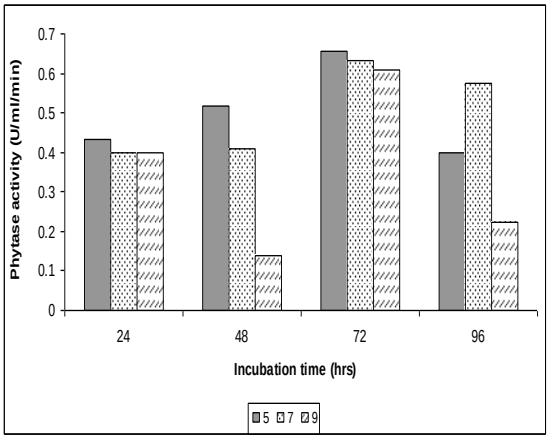

Fig. 2. Effect of pH on phytase production with different time intervals

Effect of different incubation temperature on phytase production with different time intervals showed that 37ºC at 72h was the optimum temperature for optimum phytase production (Table.3 & Fig.3).

Table-3 Effect of different incubation temperature on phytase production with different time intervals

Temperature	Time Intervals			
	24	48	72	96
	Phytase Activity (U/ml)			
27ºC	0.434	0.399	0.575	0.422
37ºC	0.434	0.422	0.657	0.551
47ºC	0.422	0.44	0.442	0.316

This is the mesophilic temperature and above this temperature the phytase production was decreased which may probably be due to cell death. Supporting the present study, It was reported that, the phytase production by *Rhizopus oligosporus* was maximum at 30ºC [11]. Similar results on phytase production by *Aspergillus_ficuum* TUB F-1165 was observed as maximum at 30ºC [12].

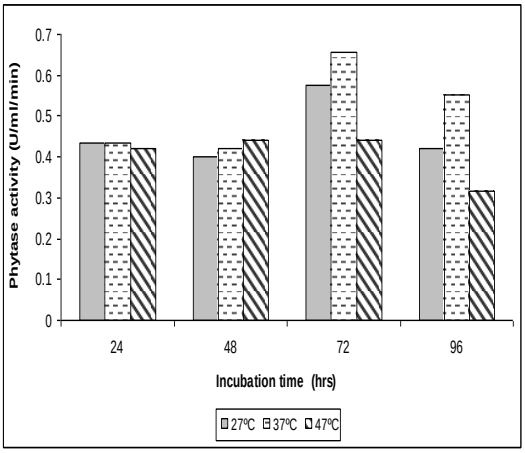

Fig.3. Effect of different incubation temperature on phytase production with different time intervals

Effect of different carbon sources on phytase production at different time intervals resulted that all the carbon sources produced considerable amount of phytase and it was specifically high at sucrose. For all the carbon sources used in the study phytase production was maximum at 72 hours of incubation (Table.4 & Fig. 4). Sucrose is a disaccharide it evident as a good energy source for phytase production by the organism. This was supported by the studies in *A.niger*, where the phytase production was heavily induced by sucrose in *A. niger* [13].

Table. 4. Effect of different carbon sources on phytase production at different time intervals

Carbon sources	Time Intervals			
	24	48	72	96
	Phytase Activity (U/ml)			
Maltose	0.493	0.516	0.704	0.496
Lactose	0.457	0.692	0.724	0.469
Sucrose	0.457	0.516	0.739	0.410
Glucose	0.481	0.522	0.727	0.469

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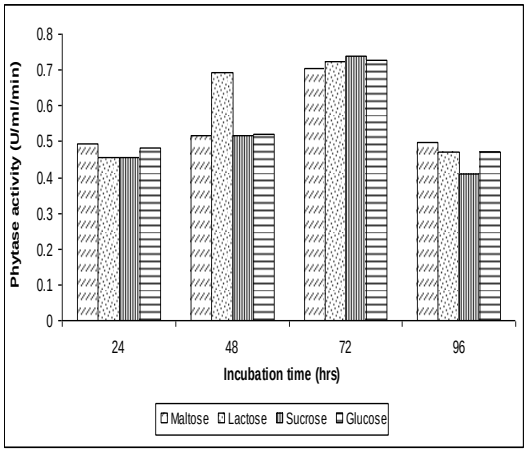

Fig. 4. Effect of different carbon sources on phytase production at different time intervals

Nitrogen sources are important nutrients for phytase production and the effect of different nitrogen sources at different time intervals resulted in higher rate of phytase production with ammonium sulphate as a nitrogen source at 72 hours of incubation (Table.5 & Fig 5).

Table.5. Effect of different nitrogen sources on phytase production at different time intervals

Nitrogen Sources	Time Intervals			
	24	48	72	96
	Phytase Activity (U/ml)			
Ammonium nitrate	0.551	0.469	0.692	0.516
Ammonium sulphate	0.457	0.516	0.751	0.540
Malt extract	0.516	0.540	0.633	0.493
Yeast extract	0.469	0.540	0.739	0.457

Next to ammonium sulphate yeast extract was also produced considerable amount of phytase. Supporting the present study *Saccharomyces cerevisiae* CY are reported to produce maximum phytase in ammonium sulphate [8]. Similarly, it was alos reported that the ammonium sulphate was a significant nutrient for maximum phytase production by

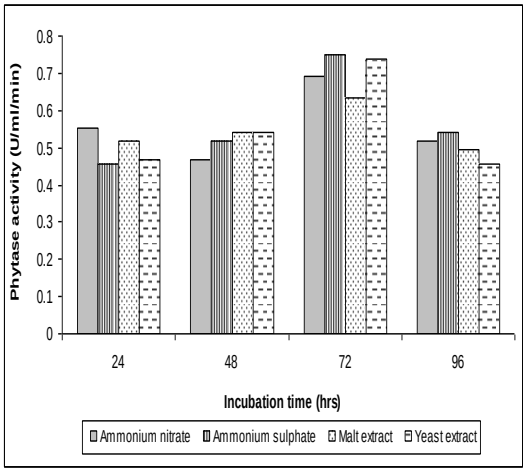

Fig.5. Effect of different nitrogen sources on phytase production at different time intervals

Aspergillus ficuum NRRL3135 [14]. Likewise, the phytase production by *Mucor indicus* MTCC 6333 was reported as maximum in the presence of ammonium phosphate as a nitrogen source [10].

Phosphates have been reported to be either a repressor or a inducer of phytase production in different microorganisms. Two different inorganic phosphates namely Tricalcium phosphate and Sodium dihydrogen ortho phosphate were screened for phytase production. The results showed that, the phytase production was maximum in Tricalcium phosphate at 72 hours of incubation (Table-6 & Fig. 6).

Table. 6. Effect of inorganic phosphates as phosphate source on phytase production at different time intervals.

Phosphate Sources	Time Intervals			
	24	48	72	96
	Phytase Activity (U/ml)			
Tricalcium phosphate	0.457	0.516	0.751	0.422
Sodium phosphate	0.493	0.54	0.727	0.446

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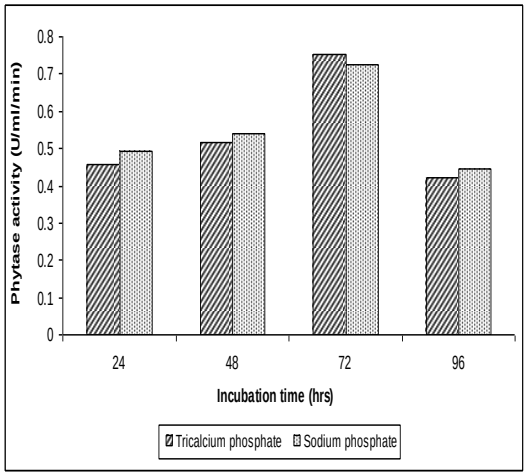

Fig.6. Effect of inorganic phosphates as phosphate source on phytase production at different time intervals.

In agreement with the present study phytase production by *Aspergillus niger* NCIM 1207 increased by supplementary phosphates [15]. Similarly, it was reported that the phytase production by *Thermoascus aurantiacus* was increased with increase in concentration of potassium dihydrogen orthophosphate [16].

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