Optimization of Physical and Nutritive Parameters for Phytase Production from Fermented Pentaclethra Macrophylla (Obj 2 and 3)

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DOI: https://doi.org/10.51584/IJRIAS.2024.905015

Received: 26 April 2024; Accepted: 06 May 2024; Published: 05 June 2024

ABSTRACT

Producing natural phytase without any health or environmental challenge targeted the main focus of many researchers. Many studies focused on fungal phytase as an extracellular phytase, but due to substrate specificity, resistance to proteolysis and catalytic efficiency, bacterial phytase is now better alternative to fungal phytase. This study was undertaken to optimize the physical and nutritive parameters for phytase production from fermented *Pentaclethra macrophylla*. Prepared raw and fermented Oil bean seeds were randomly purchased from various markets in Awka metropolis, Anambra State, Nigeria. The fermented Oil bean seeds were screened for phytase-producing bacteria using standard plate technique The amount of phytase produced was optimized at varying pH, temperature, incubation time, nitrogen, and carbon sources while nutritive contents were determined using gravimetric. The data generated from the study were analyzed statistically using one way Analysis of Variance (ANOVA) and Student's "t" test. The study revealed significant ($P < 0.05$) reduction in carbohydrate content, non-significant ($P > 0.05$) reduction in ash and fiber contents, increased in moisture and proteins contents, and significant ($P < 0.05$) reduction in phytic acid during fermented. There was maximum production of phytase at pH 7.0, growth temperature of 30^{o}C, glucose, and ammonium sulphate as carbon and nitrogen sources after 4 days. Therefore, *Pentaclethra macrophylla* contains essential nutrients, and phytase is produced at optimum conditions.

INTRODUCTION

Pentaclethra macrophylla is also known as Oil bean due to its high content of oil, nutritional, and anti-nutritional components (Kalsi *et al.*, 2016). Its nutritive contents had led to increase intake especially in the Eastern part of Nigeria, where it is cultivated in abundant. In addition to lack of protein and energy, a major cause of under-nutrition is inadequate micronutrients such as vitamin A, iodine, iron, and zinc (Dan *et al.*, 2017).

These nutritionally-related problems are more prevalent among the resource-poor living in the rural areas and poor city dwellers, because they cannot afford balanced diets. Zinc plays an important role in insulin action, carbohydrate and protein metabolism (Priyodip *et al.*, 2017). The administration of zinc, magnesium, selenium, vitamin A, and vitamin E improves tissue response to insulin and increases the efficacy of drugs which act through this pathway (Klosowki *et al.*, 2018).

The consumption of foods rich in iron, zinc, selenium and other vital micronutrients is, therefore, important to the human wellbeing. Legumes and Oil bean seeds have been noted as a major source of protein, complex carbohydrates and minerals in developing countries. Legumes and Oil bean seeds are also low in fat, do not have cholesterol, and are rich in other dietary components such as dietary fibre, which is important in weight

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume IX Issue V May 2024

management and in regulating blood cholesterol and sugar (Kammoun *et al*., 2012).

Research had revealed that Oil bean also contains anti-nutritive compound known as phytate, which can be ameliorated by phytase production (Singh, 2014; Kalsi *et al*., 2016). Also, certain bacterial species had been shown to produce phytase when cultured on Phytate Screening Medium (PSM), which are basically inorganic salts and phyate compound.

Some of the microorganisms that have phytase-producing potentials had been reported (Zhuo *et al*., 2014; Jain and Singh, 2017; Rizmanuddin *et al*., 2023). Fungi are known for their ability to produce extracellular-enzymes compared to intracellular-enzymes produced by bacteria and yeast cells, and this makes fungal cells attractive for large-scale production of enzymes (Roy *et al*., 2014; Atoyebi *et al*., 2017).

Phytase production is highly influenced by certain factors such as temperature, incubation time, carbon source, nitrogen source, pH etc. These factors are critically controlled during phytase production because the producers of the enzyme are selective in terms of growth conditions as well as carbon and energy sources (Badau *et al*., 2013; Samtiya *et al*., 2020).

Several researchers had worked on the effect of phytase produced from legumes fermentation on phytic acid such as Yellavila *et al*. (2015), Jain and Singh (2017) and Ogbmudia *et al*. (2017) but little information had been documented on the optimization of physical and nutritive parameters for phytase production from fermented *Pentaclethra macrophylla*. Hence, the aim of this study is to evaluate the optimization of physical and nutritive parameters for phytase production from fermented *Pentaclethra macrophylla*. The result obtained in this study would be essential in optimum fermentation of *Pentaclethra macrophylla* for enhancement of nutritional content.

MATERIALS AND METHODS

Screening for Phytase Producing Bacterial Isolates from Fermented African Oil Bean Seeds

This was carried out using the method described in the study published by Akter *et al*. (2016), Kalsi *et al*. (2016), Mogal *et al*. (2017) and Matrol *et al*. (2023).

Qualitative determination of phytase: Ten grams of fermented *Pentaclethra macrophylla* seeds was weighed into 250 mL of conical flask (Pyrex), about 40 mL of normal saline (0.85% NaCl). This was shake thoroughly and then made up to 100 mL using the normal saline. Then 0.1 mL of this suspension was plated onto phytase screening medium (PSM) (1.5%glucose, 0.5% (NH4)2SO4, 0.05% KCl, 0.01% MgSO4.7H2O, 0.01% NaCl, 0.01% CaCl2.2H2 O, 0.001% FeSO4, 0.001% MnSO4, 3.5 % of agar agar, pH 6.5 with 0.5% sodium phytate (Sigma)). The colonies exhibiting zones of clearance (translucent areas) were selected and streaked on phytase screening medium containing sodium phytate as substrate. The clearance zone and colony diameter were measured after 2-5 days of incubation at 37°C. The phytase index was determined as follows:

$$\text{Phytase Index} = \frac{\text{Diameter zone of inhibition} - \text{Diameter of the colony}}{\text{Diameter of the colony}}$$

Optimization of Physical and Nutritional Parameters for Phytase Production

This was carried out using the method described in the study published by Akter *et al*. (2016), Kalsi *et al*. (2016), Mogal *et al*. (2017) and Matrol *et al*. (2023). In order to study the definite growth pattern of the isolates and optimum phytase production, the temperature, pH, incubation time, carbon and nitrogen

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume IX Issue V May 2024

sources were optimized for each strain.The pure bacterial isolates were sub cultured in phytase medium (PM) and nutrient agar (BIOTECH). This later sub cultured in nutrient broth containing 0.5% sodium phytate, and incubated at room temperature (30±2^{o}C) for 24 h.

pH optimization: Then 1.0 mL of this suspension was inoculated onto phytase medium (PM) containing 1.5%glucose, 0.5% (NH4)2SO4, 0.05% KCl, 0.01% MgSO4.7H2O, 0.01% NaCl, 0.01% CaCl2.2H2 O, 0.001% FeSO4, 0.001% MnSO4, with 0.5% sodium phytate (Sigma)) adjusted at varying pH range (4.0, 5.0, 6.0, 7.0, 9.0, 10.0) prepared in triplicates, and incubated at room temperature (30±2^{o}C) for 96 h. The filtrates were collected and phytase activities were assayed by mixing 100 µl enzyme and 900 µl 0.1 Msodium acetate-acetic acid buffer (100 mM, pH 5.5) containing sodium phytase (2mM) and incubated at 50^{0}C for 15 min. A 500 µl of 10 % (w/v) trichloroacetic acid (TCA) was used to stop the reaction. Then, 1 mLof colour reagent containing (4 volumes of ammonium molybdate (2.5 % w/v) in sufuric acid (5.5 % v/v)and 1 volume of ferrous sulfate (2.5 % w/v) was added to the mixture and centrifuged at 12,000 xg for 5 min at 4^{0}C. The clear upper layer was collected and incubated at 28^{0}C for 15 min. The absorbance was measured at 700 nm. The enzyme production was quantified by measuring the amount of liberated phosphate from sodium phytase. One unit of phytase produces 1 µM of inorganic phosphate per minute at 50^{0}C. The K2HPO4 (1-500 µM) was used to prepare a standard curve for comparison of the results.

Time course for enzyme production: Then 1.0 mL of this suspension was inoculated onto phytase medium (PM) containing 1.5%glucose, 0.5% (NH4)2SO4, 0.05% KCl, 0.01% MgSO4.7H2O, 0.01% NaCl, 0.01% CaCl2.2H2 O, 0.001% FeSO4, 0.001% MnSO4 with 0.5% sodium phytate (Sigma)) adjusted at optimum pH prepared in triplicates, and incubated at room temperature (30±2^{o}C) for varying enzyme production time course (1, 2, 3 and 4 days). The filtrates were collected and phytase activities were assayed by mixing 100 µl enzyme and 900 µl 0.1 M sodium acetate-acetic acid buffer (100 mM, pH 5.5) containing sodium phytase (2mM) and incubated at 50 ^{0}C for 15 min. A 500 µl of 10 % (w/v) trichloroacetic acid (TCA) was used to stop the reaction. Then, 1 mL of colour reagent containing (4 volumes of ammonium molybdate (2.5 % w/v) in sufuric acid (5.5 % v/v) and 1 volume of ferrous sulfate (2.5 % w/v) was added to the mixture and centrifuged at 12,000 xg for 5 min at 4 ^{0}C. The clear upper layer was collected and incubated at 28 ^{0}C for 15 min. The absorbance was measured at 700 nm. The enzyme production was quantified by measuring the amount of liberated phosphate from sodium phytase. One unit of phytase produces 1 µM of inorganic phosphate per minute at 50 ^{0}C. The K2HPO4 (1-500 µM) was used to prepare a standard curve for comparison of the results.

Temperature optimization: Then 1.0 mL of this suspension was inoculated onto phytase medium (PM) containing 1.5%glucose, 0.5% (NH4)2SO4, 0.05% KCl, 0.01% MgSO4.7H2O, 0.01% NaCl, 0.01% CaCl2.2H2 O, 0.001% FeSO4, 0.001% MnSO4 with 0.5% sodium phytate (Sigma)) adjusted at optimum pH prepared in triplicates, and incubated at varying temperatures (25^{o}C, 30^{o}C, 35^{o}C,50^{o}C) for optimum enzyme production time course. The filtrates were collected and phytase activities were assayed by mixing 100 µl enzyme and 900 µl 0.1 M sodium acetate-acetic acid buffer (100 mM, pH 5.5) containing sodium phytase (2mM) and incubated at 50 ^{0}C for 15 min. A 500 µl of 10 % (w/v) trichloroacetic acid (TCA) was used to stop the reaction. Then, 1 mL of colour reagent containing (4 volumes of ammonium molybdate (2.5 % w/v) in sufuric acid (5.5 % v/v) and 1 volume of ferrous sulfate (2.5 % w/v) was added to the mixture and centrifuged at 12,000 xg for 5 min at 4 ^{0}C. The clear upper layer was collected and incubated at 28 ^{0}C for 15 min. The absorbance was measured at 700 nm. The enzyme production was quantified by measuring the amount of liberated phosphate from sodium phytase. One unit of phytase produces 1 µM of inorganic phosphate per minute at 50 ^{0}C. The K2HPO4 (1-500 µM) was used to prepare a standard curve for comparison of the results.

Effect of nitrogen sources on phytase enzyme production: Then 1.0 mL of this suspension was inoculated onto phytase medium (PM) containing 1.5% of glucose, 0.5% of varying nitrogen sources [(NH4)2SO4,

peptone, $NaN0_3$, $KN0_3$], 0.05% KCl, 0.01%, 0.01% NaCl, 0.01% CaCl2.2H2 O, 0.001% FeSO4, 0.001% MnSO4 with 0.5% sodium phytate (Sigma)) adjusted at optimum pH prepared in triplicates, and incubated at optimum temperature for optimum enzyme production time course. The filtrates were collected and phytase activities were assayed by mixing 100 µl enzyme and 900 µl 0.1 M sodium acetate-acetic acid buffer (100 mM, pH 5.5) containing sodium phytase (2mM) and incubated at 50 ^{0}C for 15 min. A 500 µl of 10 % (w/v) trichloroacetic acid (TCA) was used to stop the reaction. Then, 1 mL of colour reagent containing (4 volumes of ammonium molybdate (2.5 % w/v) in sufuric acid (5.5 % v/v) and 1 volume of ferrous sulfate (2.5 % w/v) was added to the mixture and centrifuged at 12,000 xg for 5 min at 4 ^{0}C. The clear upper layer was collected and incubated at 28 ^{0}C for 15 min. The absorbance was measured at 700 nm. The enzyme production was quantified by measuring the amount of liberated phosphate from sodium phytase. One unit of phytase produces 1 µM of inorganic phosphate per minute at 50 ^{0}C. The K_2HPO4 (1-500 µM) was used to prepare a standard curve for comparison of the results.

Effect of simple and complex carbon sources on phytase enzyme production: Then 1.0 mL of this suspension was inoculated onto phytase medium (PM) containing 1.5% of varying carbon sources (glucose, sucrose, lactose, starch maltose), 0.5% $(NH4)_2SO_4$, 0.05% KCl, 0.01% $MgSO_4.7H_2O$, 0.01% NaCl, 0.01% $CaCl_2.2H_2O$, 0.001% $FeSO_4$, 0.001% $MnSO_4$ with 0.5% sodium phytate (Sigma)) adjusted at optimum pH prepared in triplicates, and incubated at optimum temperature for optimum enzyme production time course. The filtrates were collected and phytase activities were assayed by mixing 100 µl enzyme and 900 µl 0.1 M sodium acetate-acetic acid buffer (100 mM, pH 5.5) containing sodium phytase (2mM) and incubated at 50 ^{0} C for 15 min. A 500 µl of 10 % (w/v) trichloroacetic acid (TCA) was used to stop the reaction. Then, 1 mL of colour reagent containing (4 volumes of ammonium molybdate (2.5 % w/v) in sufuric acid (5.5 % v/v) and 1 volume of ferrous sulfate (2.5 % w/v) was added to the mixture and centrifuged at 12,000 xg for 5 min at 4 ^{0}C. The clear upper layer was collected and incubated at 28 ^{0}C for 15 min. The absorbance was measured at 700 nm. The enzyme production was quantified by measuring the amount of liberated phosphate from sodium phytase. One unit of phytase produces 1 µM of inorganic phosphate per minute at 50 ^{0}C. The K2HPO4 (1-500 µM) was used to prepare a standard curve for comparison of the results.

Phytase production at optimum conditions: Then 1.0 mL of this suspension was inoculated onto phytase medium (PM) containing 1.5% of varying carbon sources glucose, 0.5% (NH4)2SO4, 0.05% KCl, 0.01% MgSO4.7H2O, 0.01% NaCl, 0.01% CaCl2.2H2 O, 0.001% FeSO4, 0.001% MnSO4 with 0.5% sodium phytate (Sigma)) adjusted at pH 7.0 prepared in triplicates, and incubated at 30^{0}C for 96 h. The filtrates were collected and phytase activities were assayed by mixing 100 µl enzyme and 900 µl 0.1 M sodium acetate-acetic acid buffer (100 mM, pH 5.5) containing sodium phytase (2mM) and incubated at 50 ^{0}C for 15 min. A 500 µl of 10 % (w/v) trichloroacetic acid (TCA) was used to stop the reaction. Then, 1 mL of colour reagent containing (4 volumes of ammonium molybdate (2.5 % w/v) in sufuric acid (5.5 % v/v) and 1 volume of ferrous sulfate (2.5 % w/v) was added to the mixture and centrifuged at 12,000 xg for 5 min at 4 ^{0} C. The clear upper layer was collected and incubated at 28 ^{0}C for 15 min. The absorbance was measured at 700 nm. The enzyme production was quantified by measuring the amount of liberated phosphate from sodium phytase. One unit of phytase produces 1 µM of inorganic phosphate per minute at 50 ^{0}C. The K2HPO4 (1-500 µM) was used to prepare a standard curve for comparison of the results.

RESULTS AND DISCUSSION

Optimization of Physical and Nutritional Parameters for Phytase Production

The effects of pH, incubation time, and growth temperature on phytase production are shown in Tables 1, 2, and 3. The study revealed that phytase production was observed at alkaline pH than acidic pH but showed optimal production when the pH was neutral (pH7) as shown in Table 5. It was also observed that isolates A3 and B4 recorded their highest phytase production when grown in a medium with pH =10, isolate B4

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume IX Issue V May 2024

recorded the highest phytase production at pH = 7. The study also revealed that MO1 significantly ($P < 0.05$) recorded the highest phytase production at pH = 7 when compared to isolates A3, A5, B4, and MO2, followed by isolate B2 as shown in Table 1. The study also revealed that phytase production increased daily and recorded the highest production after 4 days as shown in Table 2. There was significant ($P < 0.05$) increase when compared to day 1 and day 4 but the increase in day 2 and day 3 was not significant ($P > 0.05$) when compared to that of day 4, and slight increase were observed after day 3. The study also showed that isolates MO1 and B2 recorded the highest phytase production after day 4. The study also revealed that phytase production was maximally produced when the growth temperature was $30^{o}C$, and isolates B2 and MNO1 recorded the highest phytase production. The study also revealed that isolate B4 showed maximal production of phytase at growth temperature of $50^{o}C$ as shown in Table 3. The effects of nitrogen source and carbon source on phytase production are shown in Tables 8 and 9. The study revealed that ammonium sulphate $(NH4)_2SO_4$ showed the highest production of phytase, followed by potassium nitrate (KNO_3), Sodium nitrate ($NaNO_3$) whereas peptone showed the least production of phytase but there was no significant ($P > 0.05$) difference among the sources of nitrogen used in this study as shown in Table 4. Also, isolate MO1 showed the highest production of phytase, followed by isolates B2, MO2, B4, A3, and A5. Among the carbon sources, glucose recorded the highest phytase production, followed by maltose, lactose, sucrose, and starch as shown in Table 5. There was significant difference ($P < 0.05$) in phytase produced by glucose when compared to that produced by starch, but no significant difference ($P > 0.05$) to phytase produced by maltose, lactose, and sucrose. Also, isolate MO1 showed the highest production of phytase, followed by B2, MO2, B4, A3, and A5 as shown in Table 5.

The absorbance, concentrations, and enzyme activities of phytases generated from the bacterial isolates at optimal pH, temperature, incubation time, nitrogen source and carbon source are shown in Table 6. The study revealed that phytase produced at this stage was higher when compared with phytase produced at other conditions. Isolate M01 showed the maximum production of phytase, followed by B2, MO2, B4, A3, and A5 as shown in Table 6.

Table 1: Effect of pH on phytase production from the isolates

pH		A3	A5	B2	B4	MO1	MO2
4	ABS	0.306	0.269	0.514	0.366	0.610	0.412
	C(mmol/ml	2.701	2.323	4.824	3.315	5.810	3.785
	EA(change in mmol/ml.mm)	5.403	4.646	9.656	6.630	11.620	7.571
6	ABS	0.294	0.256	0.588	0.301	0.667	0.313
	C(mmol/ml	2.579	2.190	5.585	2.650	6.393	2.773
	EA(change in mmol/ml.mm)	5.157	4.380	11.170	5.301	12.785	5.546
7	ABS	0.259	0.242	0.654	0.301	0.874	0.441
	C(mmol/ml	2.221	2.047	6.260	2.650	8.509	4.082
	EA(change in mmol/ml.mm)	4.442	4.094	12.519	5.301	17.018	8.164
9	ABS	0.288	0.283	0.654	0.313	0.660	0.360
	C(mmol/ml	2.517	2.466	6.260	2.733	6.321	3.254
	EA(change in mmol/ml.mm)	5.035	4.933	12.519	5.546	12.642	6.507
10	ABS	0.264	0.264	0.575	0.514	0.624	0.351
	C(mmol/ml	2.274	2.274	5.452	4.824	5.953	3.162
	EA(change in mmol/ml.mm)	4.544	4.544	10.904	9.656	11.906	6.323

ABS = Absorbance; C = Concentration; EA = Enzyme Activity

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Table 2: Effect of incubation time on phytase production from the bacterial isolates

Day		A3	A5	B2	B4	MO1	MO2
1	ABS	0.348	0.288	0.660	0.518	0.717	0.585
	C(mmol/ml	3.131	2.517	6.321	4.869	6.904	5.554
	EA(change in mmol/ml.mm)	6.262	5.035	12.642	9.738	13.808	11.108
2	ABS	0.743	0.525	0.840	0.724	0.848	0.848
	C(mmol/ml	7.171	4.941	8.162	6.975	8.243	8.243
	EA(change in mmol/ml.mm)	14.339	9.881	16.323	13.951	16.487	16.487
3	ABS	0.768	0.585	0.905	0.840	0.962	0.900
	C(mmol/ml	7.425	5.554	8.826	8.162	9.409	8.775
	EA(change in mmol/ml.mm)	14.851	11.108	17.652	16.323	18.818	17.550
4	ABS	0.784	0.724	0.987	0.962	1.012	0.979
	C(mmol/ml	7.589	6.975	9.665	9.409	9.920	9.583
	EA(change in mmol/ml.mm)	15.178	13.951	19.329	18.818	19.840	19.166

ABS = Absorbance; C = Concentration; EA = Enzyme Activity

Table 3: Effect of temperature on phytase production from the bacterial isolates

Temperature		A3	A5	B2	B4	MO1	MO2
25^{o}C	ABS	0.344	0.251	0.905	0.703	1.014	0.900
	C(mmol/ml	3.090	2.139	8.826	6.761	6.941	8.775
	EA(change in mmol/ml.mm)	6.180	4.278	17.652	13.521	19.881	17.550
30 ^{o}C	ABS	0.840	0.716	0.988	0.962	1.019	0.979
	C(mmol/ml	8.162	6.894	9.675	9.409	9.992	9.583
	EA(change in mmol/ml.mm)	16.323	13.787	19.350	18.818	19.984	19.166
35 ^{o}C	ABS	0.703	0.696	0.900	0.716	0.962	0.840
	C(mmol/ml	6.761	6.689	8.775	6.894	9.409	8.162
	EA(change in mmol/ml.mm)	13.521	13.378	17.550	13.787	18.818	16.323
50 ^{o}C	ABS	0.696	0.987	0.985	1.012	0.951	0.397
	C(mmol/ml	6.689	9.665	9.644	9.920	9.297	3.632
	EA(change in mmol/ml.mm)	13.378	19.329	19.218	19.840	18.593	7.2664

ABS = Absorbance; C = Concentration; EA = Enzyme Activity

Table 4: Effect of nitrogen source on phytase production from the bacterial isolates

Nitrogen Source		A3	A5	B2	B4	MO1	MO2
Peptone	ABS	0.344	0.251	0.905	0.703	1.014	0.900
	C(mmol/ml	3.090	2.139	8.826	6.761	6.941	8.775
	EA(change in mmol/ml.mm)	6.180	4.278	17.652	13.521	19.881	17.550
$(NH4)_2SO_4$	ABS	0.840	0.716	0.988	0.962	1.019	0.979
	C(mmol/ml	8.162	6.894	9.675	9.409	9.992	9.583

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	EA(change in mmol/ml.mm)	16.323	13.787	19.350	18.818	19.984	19.166
$NaNO_3$	ABS	0.703	0.696	0.900	0.716	0.962	0.840
	C(mmol/ml	6.761	6.689	8.775	6.894	9.409	8.162
	EA(change in mmol/ml.mm)	13.521	13.378	17.550	13.787	18.818	16.323
KNO_3	ABS	0.696	0.987	0.985	1.012	0.951	0.397
	C(mmol/ml	6.689	9.665	9.644	9.920	9.297	3.632
	EA(change in mmol/ml.mm)	13.378	19.329	19.218	19.840	18.593	7.2664

ABS = Absorbance; C = Concentration; EA = Enzyme Activity

Table 5: Effect of carbon source on phytase production from the bacterial isolates

Carbon Source		A3	A5	B2	B4	MO1	MO2
Starch	ABS	0.593	0.563	0.694	0.693	0.694	0.639
	C(mmol/ml	5,636	5.329	6.669	6.658	6.669	6.106
	EA(change in mmol/ml.mm)	11.272	10.658	13.337	13.317	13.337	12.213
Sucrose	ABS	0.582	0.718	0.554	0.587	0.728	0.726
	C(mmol/ml	5.524	6.914	5.237	5.575	7.016	6.996
	EA(change in mmol/ml.mm)	11.047	13.828	10.474	11.149	14.033	13.992
Lactose	ABS	0.628	0.805	0.587	0.580	0.745	0.728
	C(mmol/ml	5.994	7.804	5.575	5.503	7.190	7.016
	EA(change in mmol/ml.mm)	11.988	15.607	11,149	11.006	14.380	14.033
Maltose	ABS	0.558	0.805	0.726	0.614	0.840	0.549
	C(mmol/ml	5.278	7.804	6.996	5.851	8.162	5.186
	EA(change in mmol/ml.mm)	10.556	15.607	13.992	11.701	16.323	10.372
Glucose	ABS	0.820	0.808	0.979	0.840	1.014	0.962
	C(mmol/ml	7.957	7.834	9.583	8.162	9.941	9.409
	EA(change in mmol/ml.mm)	15.914	15.669	19.166	16.323	19.881	18.818

ABS = Absorbance; C = Concentration; EA = Enzyme Activity

Table 6: Phytase production at optimal pH, temperature, incubation time, carbon and nitrogen sources from the bacterial isolates

Bacterial Isolate	ABS	Conc (m.mol/mL)	Enzyme Activity(change in m.mol/mL.min
A3	0.847	8.233	16.466
A5	0.840	8.162	16.323
B2	1.014	9.941	19.881
B4	0.979	9.583	19.166
MO1	1.019	9.992	19.984
MO2	0.987	9.665	19.329

The production of phytase from the bacterial isolates at low pH in the present study corroborated with the findings of many researchers (Eze *et al*., 2014; Singh, 2014; Wulandari *et al*., 2015; Priyodip *et al.*, 2017; Rizwanuddin *et al*., 2023) who reported significant production of phytase at low pH but disagrees with

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume IX Issue V May 2024

Zailan *et al*. (2021) who reported significant production of production of phytase at high pH. In the present study, optimum secretion of phytase was detected when the pH of the secretion medium is 7.0 for those bacterial isolates (B2, MO1 and MO2) that recorded the highest production of phytase.

The significant daily increased in phytase production recorded in the present study supported the findings of many researchers (Eze *et al*., 2014; Singh, 2014; Wulandari *et al*., 2015). The highest secretion of phytase after 4 days as observed in the present study could be attributed to high and potent secretion of phytase that can withstand some environmental conditions. Similar finding was reported by Singh (2014) and Rizwanuddin *et al*. (2023).

The significant production of phytase from the bacterial isolates from fermented African Oil seeds at varying temperature was reported by many researchers (Sasirekha *et al.,* 2012; Eze *et al.,* 2014; Suleimenova *et al.,* 2016; Zailan *et al*., 2021; Asad *et al.,* 2022). The maximal production of phytase by the bacterial isolates (B2 and MO1) when the growth temperature was 30^{o}C could be attributed to the fact that this temperature favours the growth of these bacterial isolates and supported the extracellular secretion of phytase from these bacterial isolates.

Eze *et al*. (2014) and Suleimenova *et al*. (2016) reported significant and highest secretion of phytase from some microorganisms at 30^{o}C. The secretion of phytase from the bacterial isolates using different nitrogen sources ($(NH4)_2SO_4$, KNO_3, $NaNO_3$ and peptone) supported the findings of many researchers (Sasirekha *et al.,* 2012; Eze *et al.,* 2014; Suleimenova *et al.,* 2016; Zailan *et al*., 2021; Asad *et al.,* 2022). In the present study, ammonium sulphate $(NH4)_2SO_4$) recorded the highest production of phytase, and this could be attributed to the fact that the test isolates were able to utilize $(NH4)_2SO_4$ as sole source of nitrogen more than other nitrogen sources. This finding agrees with the findings of Suleimenova *et al.* (2016) and Asad *et al.* (2022) but disagrees with Sasirekha *et al.* (2012) and Zailan *et al*. (2021) who reported the highest production of phytase from yeast extract and ammonium nitrate, respectively.

The production of phytase from bacterial isolates using different carbon sources (glucose, maltose, lactose, sucrose and starch) supported the findings of many researchers (Sasirekha *et al.,* 2012; Balogun, 2013; Eze *et al*., 2014; Oyeleke *et al*., 2014; Suleimenova *et al.,* 2016; Zailan *et al*., 2021; Asad *et al.,* 2022). The highest production of phytase from the bacterial isolates when the source of carbon was glucose could be attributed to the ability of the implicated bacterial isolates to utilize glucose as sources tested in the study. Similar carbon source (glucose) was reported by Sasirekha *et al.* (2012), Eze *et al.* (2014) and Zailan *et al*. (2021). Other researchers such as Suleimenova *et al.* (2016) and Asad *et al.* (2022) reported the production of phytase when their respective carbon sources were sucrose and wheat bran, respectively. The highest and maximal secretion of phytase from the bacterial isolates when the pH, temperature, incubation time, nitrogen and carbon sources were 7.0, 30^{o}C, 4 days, ammonium sulphate and glucose could be attributed to the fact that the isolates were able to grow in their optimal growth conditions, and were able to secrete more phytase than previous conditions. Several researchers (Sasirekha *et al.,* 2012; Eze *et al*., 2014; Zailan *et al*., 2021) reported production of phytase at the optimal growth conditions of the test isolates.

CONCLUSION

The study has revealed that phytase was produced by the screened bacterial isolates when optimium conditions were provided such as the pH (7.0), temperature (30^{o}C), incubation time (4 days) , nitrogen (ammonium sulphate), and carbon sources (glucose).

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