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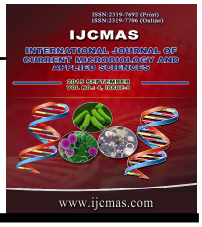

Original Research Article

Partial Purification and Characterization of Phytase from Bacteria Inhabiting the Mangroves of the Western Coast of India

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Keywords

Phytases, Environmental pollution, Animal feed, Soil amendment, Enzyme activity, Enzyme stability

A B S T R A C T

Phytases belong to a special class of phosphatase enzymes that catalyze the sequential hydrolysis of phosphate ester bonds of phytate. It is an enzyme of economical importance due to its applications as an animal feed additive, in preparation of myo-inositol phosphate, in the paper and pulp industry and in agriculture. Although there are several sources of phytase, microbial sources offer better prospects in terms of commercial production. The objective of the present study was to isolate phytase producing bacteria from mangrove soil followed by extraction and purification of the enzyme. Out of 33 colonies on wheat bran extract plates, 18 colonies showed positive for phytase production on phytase screening medium as indicated by clear zones of hydrolysis around them. Hydrolysis efficiency was calculated and 5 isolates with higher hydrolysis efficiency were investigated for phytase production. The phytase enzyme was produced using shaken flask fermentation and partially purified using ammonium sulphate precipitation and dialysis. The molecular weight of the enzyme determined using SDS-PAGE was in the range of 16–22 KDa. The partially purified enzyme exhibited an optimum temperature range of 45-55°C and retained 51% activity at 60°C. The enzyme remained stable with almost 89% residual activity till 60°C preincubation temperature. Optimum pH value of 5 was recorded and the enzyme was stable over a pH range of 2–5. The enzyme was susceptible to proteolytic denaturation due to pepsin treatment.

Introduction

Due to the growth in livestock population, increasing domestic consumption of animal products and growth of end-user companies, the Indian animal feed industry is predicted to take off in a big way in the coming decade. It is mainly restricted to dairy and poultry feed manufacturing. Feed is also required for the maintenance of laboratory animals. Ensuring quality is a major concern in feed manufacture, a fact that includes fortification of the feed with nutrients and enhancing their availability. Cereals, legumes and oilseeds that are used in animal feeds have a high (nearly 80%) of total phosphate content in the form of phytic acid or phytate (Chang *et al.*, 1977; Reddy *et al.*,

1989; Graf, 1986). Due to lack of phytases the non-ruminant monogastric animals like pigs, poultry and fish cannot utilize dietary phytic acid phosphorous (Lantzsch *et al.*, 1995). Phytate also chelates proteins and other essential minerals like calcium, iron, zinc, magnesium, manganese, copper and molybdenum in the feed acting as an anti-nutrient (Sreedevi and Reddy, 2013b). Unused phytic acid is excreted in manure. High phytic acid content in manure results in elevated levels of phosphorous in soil and eutrophication of surface water leading to environmental pollution. Supplementation of animal feed with Phytase enzyme increases bio-availability of phosphorous and reduces the negative impact to the environment. This is also economical as it reduces the need to add inorganic phosphate (Saravanamuthu, 2010; Sreedevi and Reddy, 2013a). Phytases are widespread in nature and can be derived from a host of sources including plants, animals and microorganisms. Microbial sources are more promising for the production of phytases on a commercial scale (Reddy *et al.*, 1989; Pandey *et al.*, 2001b; Nam-Soon Oh and Man-Jin In, 2009). High production capability, low cost and susceptibility to genetic manipulation are important attributes of microbial sources. Almost all microbial classes including bacteria, yeasts and fungi have been reported to produce phytase (Sreedevi and Reddy, 2013a, b). Due to several biological characteristics, such as substrate specificity, resistance to proteolysis and catalytic efficiency, bacterial phytases have considerable potential in commercial applications (Sreedevi and Reddy, 2013b).

The mangrove ecosystem although rich in organic matter is nutrient deficient especially in nitrogen and inorganic phosphates. Nutrients are made available to the plants due to recycling activity carried out by the soil microorganisms. Mangroves ecosystems exhibit a highly productive and diverse microbial community (Das *et al.*, 2013). Mangrove microorganisms have a diverse range of enzymatic activity and are capable of catalyzing various biochemical reactions with novel enzymes (Thatoi *et al.*, 2012). Very little is known about the microbial communities of mangroves and these microorganisms may have high biotechnological potential that is yet untapped (Castro *et al.*, 2014). There are very few reports of phytase producing bacteria isolated from mangroves especially in India. Mangrove ecosystems are therefore an important but less explored source of microorganisms, capable producing enzymes of industrial importance.

The present study involved the isolation of phytase producing bacteria from mangrove regions of Navi Mumbai, Maharashtra. The enzyme was extracted, partially purified and characterized in terms of thermal, pH and proteolytic treatment stability.

Materials and Methods

Isolation and screening of phytase producing bacteria

Rhizosphere soil samples were collected from mangrove regions at two sites in Navi Mumbai (Belapur and Kamothe). One gram of each soil sample was suspended in 10ml of sterile distilled water and was serially diluted 10 fold. 100µl of the dilutions were plated on wheat bran agar containing $(NH_4)2SO_4$-0.04%, $MgSO_4.7H_2O$-0.02%, casein-0.1%, KH_2PO_4-0.05%, K_2HPO_4-0.04% and agar-2%. The inoculated plates were incubated at 37°C for 1- 3 days and observed for the clear zones of hydrolysis around the colonies. This indicates production of extracellular phytase (Sreedevi and Reddy, 2013a, b; Tungala *et al.*, 2013). Further screening was done by

plating the isolates exhibiting clear zones on phytase screening medium (PSM) containing 1.5% - glucose, 0.5% - $(NH_4)_2SO_4$, 0.05% - KCl, 0.01% - $MgSO_4.7H_2O$, 0.01% - NaCl, 0.01% - $CaCl_2.2H_2O$, 0.001% - $FeSO_4$, 0.001% - $MnSO_4$, 1.5%-agar, pH 6.5 with 0.5% calcium phytate (ChemPure). Isolates showing distinct zone of clearance were tested for hydrolysis efficacy based on the diameter of zone of clearance. 5 isolates showing 50% hydrolysis efficacy were selected for further study. The positive isolates were selected and transferred to nutrient agar slants and were then stored at 4°C until use (Hosseinkhani *et al.,* 2009; Moushree Roy *et al.,* 2012; Mittal *et al.,* 2011; Sreedevi and Reddy, 2012; Imelda Joseph and Paul Raj, 2007; Mukesh Kumar *et al.,* 2011; Mishra *et al.*, 2013; Sasirekha *et al.,* 2012).

Identification of phytase producing bacterial isolates

Identification of the selected strains was carried out on the basis of morphological analysis and biochemical characterization. The strains were initially examined for cell morphologies and cell arrangement by gram staining, presence or absence of spores and capsules and motility using microscopy. The various biochemical tests carried out were Indole test, Methyl red test, Voges proskauer test, Citrate utilisation tests, Triple sugar iron utilization tests, catalase, oxidase, urease, starch hydrolysis, H_2S production and carbohydrate fermentation tests. The isolates were also tested for fermentation of various sugars like glucose, lactose, mannitol, maltose and sucrose. The morphological and biochemical tests were carried out by the techniques described in the Mackie and Mc Cartney Practical Medical Microbiology (Sreedevi and Reddy, 2013aand b; Tungala *et al.,* 2013; Mittal *et al.,* 2011; Imelda Joseph and Paul Raj, 2007; Nabil M. K. El-Toukhy *et al.,* 2013; Surya *et al.,* 2013; Collee, 2007).

Enzyme assay

Phytase activity was assayed by a modification of the Heinonen-Lahti method (1981) as described by Yoon *et al.* (1996). Isolates exhibiting a positive reaction were inoculated into the PSM broth (without agar) with the same composition as stated above and incubated at 37 ± 1°C at 120 rpm for 5 days. Broth centrifuged for 10min at 10,000 rpm. The cell-free supernatant was separated and tested for phytase activity. The reaction mixture consist of 0.8 ml acetate buffer (0.2M, pH 5.5 containing 10 mM sodium phytate) 0.2 ml of supernatant. After incubation for 30 min 37°C, the reaction was stopped by adding 1 ml of 10% tricholoroacetic acid. Assay mixture of 0.5 ml was then taken in fresh set of tubes and mixed with 4 ml of 2:1:1 v/v of acetone, 10 mM ammonium molybdate and 5 N sulfuric acid and 0.4 ml of citric acid (1M). The amount of free phosphate released was determined spectrophotometrically at 355 nm. One unit of phytase activity was defined as 1μmol of phosphate produced per min per ml of culture filtrate under the assay condition. Enzyme activity was expressed in international units (U). Blank was run without degradation of the substrate by adding the stop solution prior to the addition of the enzyme to the assay mixture. A standard graph was plotted using potassium dihydrogen phosphate with working concentration ranging from 0.04-18 mM (Sreedevi and Reddy, 2013a; Tungala *et al.,* 2013; Hosseinkhani *et al.,* 2009; Moushree Roy *et al.,* 2012; Sreedevi and Reddy, 2012; Imelda Joseph and Paul Raj, 2007; Mishra *et al.,* 2013; Sasirekha *et al.,* 2012). The protein content of the enzyme preparations was estimated by Lowry method using

Bovine serum albumin (BSA) as standard at 660 nm (Lowry *et al.,* 1951).

Enzyme purification (partial)

Partial purification of phytase enzyme was achieved by ammonium sulphate precipitation followed by dialysis. All purification steps were carried out at 4°C unless otherwise stated. Five different isolated strains were cultured in phytase production media (PPM = PSM – agar) and kept in a shaker incubator at 200 rpm at 37°C for 3-5 days. After incubation, the fermented broth was subjected to centrifugation at 10000 rpm for 10 minutes at 4°C. The cell free supernatant was collected and used as crude enzyme for purification. The enzyme extract (80–100 ml of the cell-free supernatant) was fractionated by stepwise precipitation with ammonium sulphate powder at 0–40%, 40%–60%, 60%–80%, and 80%–100% saturation. The contents were incubated overnight at 4°C. The precipitate formed in each step was collected by centrifugation at 9000 rpm for 30 minutes at 4°C, dissolved in 1 ml 0.1 M acetic acid buffer and assayed for enzyme activities. The fraction showing highest enzyme activity was desalted using a dialysis bag.

The enzyme mixture (pellet/precipitate) was transferred to dialysis bag and immersed in Tris-HCl buffer (pH 7, 10 mM) at 4°C for 24 hr. The desalted samples were subjected to SDS-PAGE to check the homogeneity of the purified enzyme. SDS-PAGE was carried out as described by Laemmli with modifications (Sreedevi and Reddy, 2013a; Tungala *et al.,* 2013; Moushree Roy *et al.,* 2012; Sasirekha *et al.,* 2012; Nabil M. K. El-Toukhy *et al.,* 2013; Surya *et al.,* 2013; Neelakanthan *et al.,* 2013; Pankaj Kumar *et al.,* 2012; Shamna *et al.,* 2102).

Molecular weight determination

The molecular weight of the partially purified enzymes of the five isolates was determined by SDS-PAGE with standard marker proteins. SDS-PAGE was carried out according to the method of Laemmli, 1970; with a 12% resolving gel and 5% stacking gel. The separated proteins on the gel were stained with Coomassie brilliant blue. To determine the molecular weight the Rf of the standard proteins as well as the purified enzyme bands were measured. Rf of standard = ratio of the distance travelled by the standard band and the distance travelled by the dye front. Rf of enzyme sample = ratio of the distance travelled by each isolate enzyme band and the distance traveled by the dye front. Standard curve of Log (Molecular Weight) of standard proteins against Rf values was plotted. Molecular weight of the sample enzyme was determined from this standard curve (Sreedevi and Reddy, 2012).

Effect of temperature on enzyme activity and stability

The temperature profile of partially purified phytase was determined in the temperature range of 15–70°C following the standard phytase assay described earlier. To study the thermal stability the partially purified enzyme was incubated at 50, 60, 70 and 80°C for 10min and cooled to 4°C. Following the standard phytase assay the residual enzyme activity was assayed.

Effect of pH on enzyme activity and stability

The pH profile of the partially purified phytase was determined by assaying enzyme activity at different pH values ranging from 2-9 in various buffers 0.1M glycine-HCl (pH 2–3); 0.1M Sodium acetate-HCl (pH 4–

7); 0.1M Tris-HCl (pH 8) and 0.1M glycine-NaOH (pH 9–11). To study the effect of pH on enzyme stability, samples of the enzyme were pre-incubated in buffers of various pH values at room temperature for 20 h and then the residual activities of the enzyme were assayed.

Effect of pepsin treatment on enzyme activity

The enzyme extract was pre-incubated for 30min at 37°C with 1000U and 3000U of Pepsin (at pH 2; 75mM glycine). This was followed by standard phytase assay and the residual enzyme activity was calculated (Sreedevi and Reddy, 2013a; Greiner and Farouk, 2007).

Results and Discussion

Isolation and identification of phytase producing strains

Rhizosphere soil from mangroves of two regions in Navi Mumbai was collected for isolation of phytase producing bacteria. A total of 33 colonies appeared on wheat bran extract agar of which 18 colonies showed positive for phytase production on PSM as indicated by the clear zones of hydrolysis around them. The identification of the 18 isolates was done based on microscopic observation of morphological characteristics and biochemical tests. Isolates were categorized in to three sets following morphological and biochemical evaluation. The first set comprised of motile, gram-positive, sporulating rods. The strains of this set were Indole test negative, Methyl Red test negative, Voges Proskauer test positive, Citrate utilisation test positive, catalase and oxidase positive. The strains exhibited starch hydrolysis and fermentation of various sugars like glucose, mannitol, maltose, sucrose, lactose and galactose with production of acid and no gas formation. Based on the characteristics the isolates of this group were placed in to the *Bacillus sp.* The second set included gram positive cocci. These strains were Indole test negative, Methyl Red test negative, Voges Proskauer test positive, Citrate utilisation test positive, catalase positive and oxidase negative. The strains exhibited starch hydrolysis and fermentation of various sugars like glucose, mannitol, maltose, sucrose, lactose and galactose with production of acid and urease negative. These characteristics point to the *Micrococcus sp*. The third set included motile gram negative rods. These strains were indole negative, H2S negative, Methyl Red test negative, Voges Proskauer test positive, Citrate utilisation test positive, catalase positive and oxidase negative. The strains also exhibited starch hydrolysis. Isolates of the third set can be placed in to the *Enterobacter sp*.

Partial purification of phytase from selected isolates

The crude enzyme samples were purified by ammonium sulphate precipitation (60-80%) and dialysis. The purified enzyme (dialyzed) of isolate 1 showed a specific activity of 5.583 U/mg for sodium phytate hydrolysis with 10.3fold purification and 72% yield (Table 1). Based on the results obtained following SDS-PAGE the molecular mass of this enzyme ranged between 16 and 22 KDa (Figure 1).

Effect of temperature and pH on phytase activity and stability

Partially purified enzyme of isolate 1 was selected to study the enzyme characteristics. The optimum temperature for phytase activity was found to be 45–55°C. The enzyme retained over 51% of its activity till 60°C, above which there was an abrupt

decrease in enzymatic activity with 38% activity retained at 65°C and almost complete loss of activity at 70°C (Figure 2). The enzyme retained almost 89% activity at 60°C pre-incubation temperature indicating thermal stability. However, there was partial (46% at 70°C) and complete (0.02% at 80°C) loss of activity at higher incubation temperatures (Figure 3). Optimum pH of the purified phytase was 5.0. Almost 93% activity was retained at pH 6 and 91% at pH 4. Beyond this range a decline of enzyme activity was observed on both sides (Figure 4). Complete enzyme stability with residual activities between 99-100% was observed over pre-incubation pH range of 2-5. Beyond pH 7 there was a rapid decline in residual activity (Figure 5).

Effect of pepsin treatment on enzyme stability

The enzyme lost most of its activity (30% residual activity) when pre-incubated with 3000U of pepsin indicating susceptibility to proteolytic denaturation (Table 2).

Statistical analysis

The results of effect of temperature and pH on phytase enzyme activity studies were statistically analyzed by one-way ANOVA followed by Post hoc Least Significant Difference (LSD) test to find out whether the mean at various temperatures and pH values varied significantly. The statistical package used is SPSS, version 19 IBM Corporation, Somers, NY, USA.

Although enzyme preparations of phytases from *Aspergillus niger, Peniophora lycii, Schizosaccharomyces pombe* and *Escherichia coli* are available commercially, phytases fulfilling all the requisites for animal feed applications have not been found in nature to date (Greiner and Konietzny, 2011). Many attempts have been made to isolate phytase producing microorganisms from soils of poultry and cattle sheds, fields of cereals and pulses, ponds, poultry faeces, gut microflora and rhizospheric soils of terrestrial plants (Shamna *et al.,* 2012; Mittal *et al.,* 2011; Khan A *et al.,* 2011, Li *et al.,* 2013). However the mangrove rhizospheric soils are relatively less explored. There are a few studies that report isolation of phytase producing and phosphate solubilizing bacteria from rhizosphere microbial community in mangroves (Imelda Joseph and Paul Raj, 2007; Dave and Modi, 2013; Zhang *et al.,* 2015; Vazquez *et al.,* 2000). These studies indicate that mangrove habitat can be a promising source of phytase producing bacteria. Of the 33 isolates 18 were screened positive for phytase production on wheat bran extract. Further selection of five of these isolates was based on efficiency of hydrolysis. These five isolates were categorized in to two groups namely *Bacillus sp* (4 isolates) and *Enterobacter sp* (1 isolate) following morphological and biochemical analysis. Till date *Bacillus sp* represents the largest class of bacteria known to produce extracellular phytase with impressive application based propeties (Greiner and Farouk, 2007; Mittal *et al.,* 2011; Singh *et al.,* 2013; Choi, Suh and Kim, 2001; Kerovuo *et al.,* 1998; Powar and Jagannathan, 1982; Shimizu, 1992). The *Enterobacter sp* also represents another important genus of bacteria besides *Bacillus* to produce phytase (Greiner and Farouk, 2007; Pandey *et al.,* 2001; Yoon *et al.,* 1996).

Partial purification of phytase was achieved by Ammonium sulphate precipitation followed by dialysis. The molecular mass of the partially purified enzymes of all five isolates ranged between 16-22 KDa that is

much lower than that described in other studies. Most of the phytases characterized thus far are monomeric enzymes with molecular mass ranging between 40–70 KDa (Konietzny and Greiner, 2002). *Bacillus subtilis and Bacillus subtilis (natto)* phytases exhibit a molecular mass between 36 and 38 KDa (Shimizu, 1992, Kerovuo *et al.,* 1998) and that of *Enterobacter sakazakii ASUIA279* showed a molecular mass of 42 KDa (Farouk, Greiner and Hussin, 2012). Since the enzyme is only partially purified as is evident from the appearance of more than one band in SDS PAGE the molecular mass values derived in this study may not be conclusive.

Of the five isolates, isolate 1 (identified as belonging to the *Enterobacter sp*) showed maximum specific activity in standard phytase assay and was selected for further determination of enzymatic parameters. Extracellular phytases have great potential as animal feed supplements. Hence novel enzyme isolates must be assayed for characteristics like; Optimum temperature, optimum pH, and thermal, pH and proteolytic stability.

The partially purified phytase in the present study had an optimum temperature range of 45-55°C and retained 51% activity at 60°C. The phytase activity was significantly higher between 45 to 55°C than that obtained at temperatures below and higher than this range ($p<0.05$). This is in accordance with the optimum temperature reported in other studies. Most microbial phytases show an optimum temperature range of 45–55°C (Kerovuo *et al.,* 2000). Phytases with high temperature optima and thermal stability are desirable in the animal feed industry since feed pelleting is generally performed at temperatures from 60 to 95°C. These should be able to resist denaturation by heat during feed processing and storage. Also the enzyme in study retained 89% activity when pre-incubated at 60°C and 46% activity at 70°C. This is comparable to the phytase purified from Malaysian water bacterium that retained 50% activity when exposed to 70°C (Greiner and Farouk, 2007). Thus enzyme in the present study is more thermostable compared to phytases from *A. niger* and *P. lycii* that showed very little residual activity after pre-incubation at 70°C (Greiner and Farouk, 2007). Maximum thermostability is exhibited by the enzyme from *E. coli* with almost 100% residual activity at 62°C and 27% residual activity after pre-incubation at 85°C (Garret *et al.,* 2004). Phytase from *Enterobacter sakazakii* ASUIA279 also showed temperature optima of 45-55°C and also retained 22% activity when pre-incubated at 90°C for 15min (Farouk *et al.,* 2012). Heat destruction of supplement enzymes due to pelleting can be overcome by spraying enzyme over feed post pelleting.

Bacillus sp phytases as well as those belonging to the *Enterobacter sp* show an optimum pH of 6.5–7.5 (Pandey *et al.,* 2001; Yoon *et al.,* 1996; Kim *et al.,* 1998). Other phytases have been reported to have a pH optimum of 4–6 (Konietzny and Greiner, 2002). In the present study maximum phytase activity was recorded at pH 5. The activity drastically declined above 6. However, reasonably good activity was seen over a pH range of 2-5 with almost 80% activity retained at pH 2. Thus the phytase can be classified as an acid phytase. The phytase activity was significantly higher in the range of 4–6 as compared to that observed beyond ($p<0.05$). Phytase belonging to *Enterobacter sakazakii* ASUIA279 exhibited a pH optimum of 4.5. Phytase in the present study showed complete stability when pre-incubated at a pH range of 2–7. The pH in the stomach of animals like pigs and fish and crop of

poultry is generally low at a value of 2–5. Thus the pH optima and pH activity profile of supplementary phytases generally are instrumental in determining their potential in these gastrointestinal compartments (Greiner and Konietzny, 2011).

The effectiveness of feed supplementation with phytase also depends on the susceptibility of the enzyme to proteolytic cleavage. In the present study the enzyme was pre-incubated with pepsin (at two different strengths) at pH 2. There was loss of activity with only 56% residual activity at 1000U and 30% residual activity at 3000U. Since the enzyme is shown to be active over a pH range from 2 to 5 this loss of activity should be due to proteolytic cleavage. Bacterial acid phytases have been shown to exhibit greater proteolytic cleavage resistance as compared to fungal acid phytases. Accordingly phytases of *E. coli*, *Klebsiella spp* and the Malaysian water bacterium retained more than 80% activity after pepsin digestion whereas phytases from *A. niger* and *P. lycii* retained only 26–42% and 2–20% activity respectively (Rodriguez *et al.,* 1999; Igbasan *et al.,* 2000; Simon and Igbasan, 2002; Kim *et al.,* 2003).

Table.1 Purification of phytase from the screened isolates

Isolates	Purification Step	Total Protein (mg/ml)	Total Activity (U/ml)	Specific Activity (U/mg)	Yield (%)	Purification fold
Isolate 1	**Crude Enzyme**	1.17	0.613	0.542		
	$(NH_4)2SO_4$ Precipitation	0.9	0.5	0.555	81	1.02
	Dialyzed	0.080	0.446	5.583	72	10.3
Isolate 2	**Crude Enzyme**	0.54	0.353	0.654		
	$(NH_4)2SO_4$ Precipitation	0.180	0.28	1.55	79	2.37
	Dialyzed	0.130	0.23	1.769	65	2.70
Isolate 3	**Crude Enzyme**	1.120	0.200	0.178		
	$(NH_4)2SO_4$ Precipitation	1.030	0.186	0.180	93	1.01
	Dialyzed	0.150	0.17	1.133	85	6.36
Isolate 4	**Crude Enzyme**	1.080	0.353	0.327		
	$(NH_4)2SO_4$ Precipitation	0.90	0.323	0.359	91	1.09
	Dialyzed	0.130	0.25	1.92	70	5.87
Isolate 5	**Crude Enzyme**	0.720	0.613	0.12		
	$(NH_4)2SO_4$ Precipitation	0.840	0.80	0.178	24.46	1.4
	Dialyzed	0.50	0.133	0.266	21	2.21

Table.2 Effect of pepsin on phytase activity

Sample	1000U Pepsin treatment Residual Activity (%) at pH2 and 37°C after 30min of incubation	3000U Pepsin treatment Residual Activity (%) at pH2 and 37°C after 30min of incubation
Isolate 1	56±1.52	30±1.5

Each value represents the mean of three responses ± SD

Figure.1 Determination of molecular weight by SDS PAGE. Lane M: Protein markers; Lane M: Protein marker; Lane 1: Dialyzed sample isolate 1; Lane 2: Dialyzed sample isolate 2; Lane 3: Dialyzed sample isolate 4 and Lane 5: Dialyzed sample isolate 5

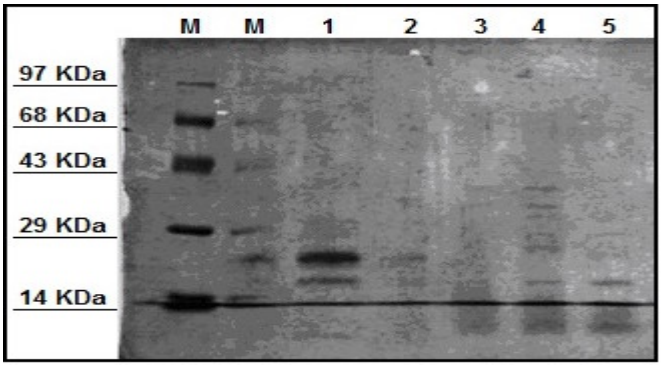


Figure.2 Effect of temperature on phytase activity

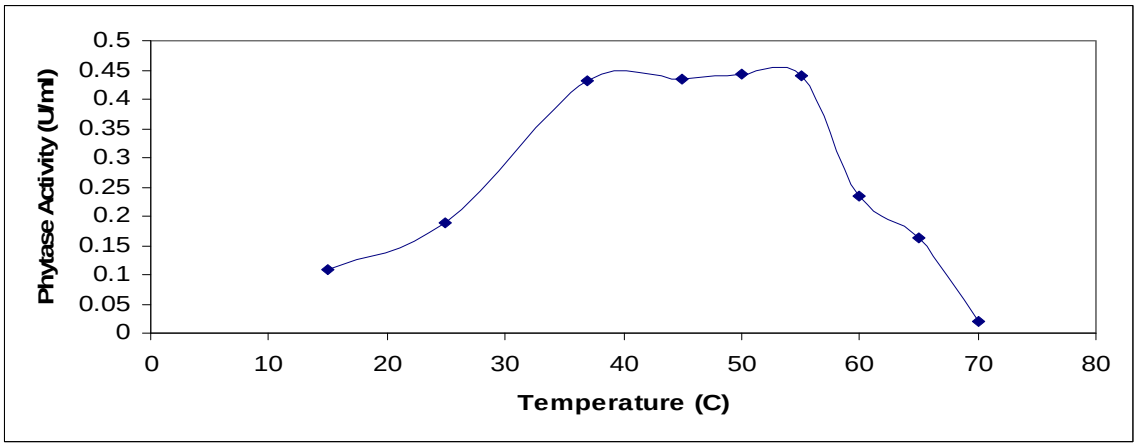


Figure.3 Effect of temperature on phytase stability

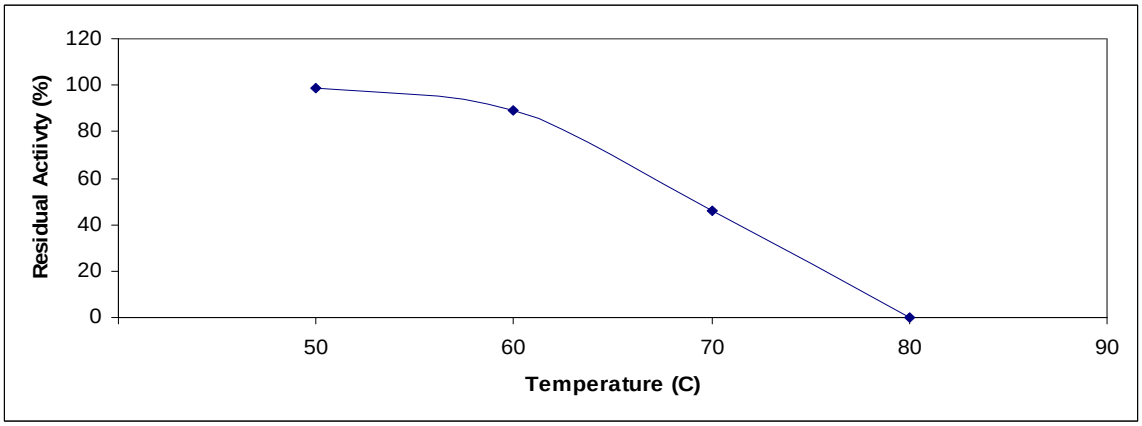


Figure.4 Effect of pH on phytase activity

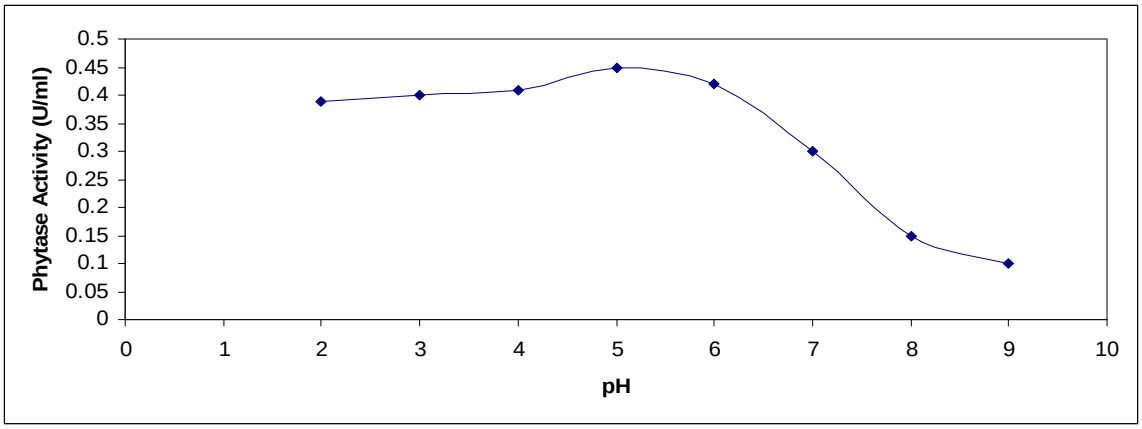
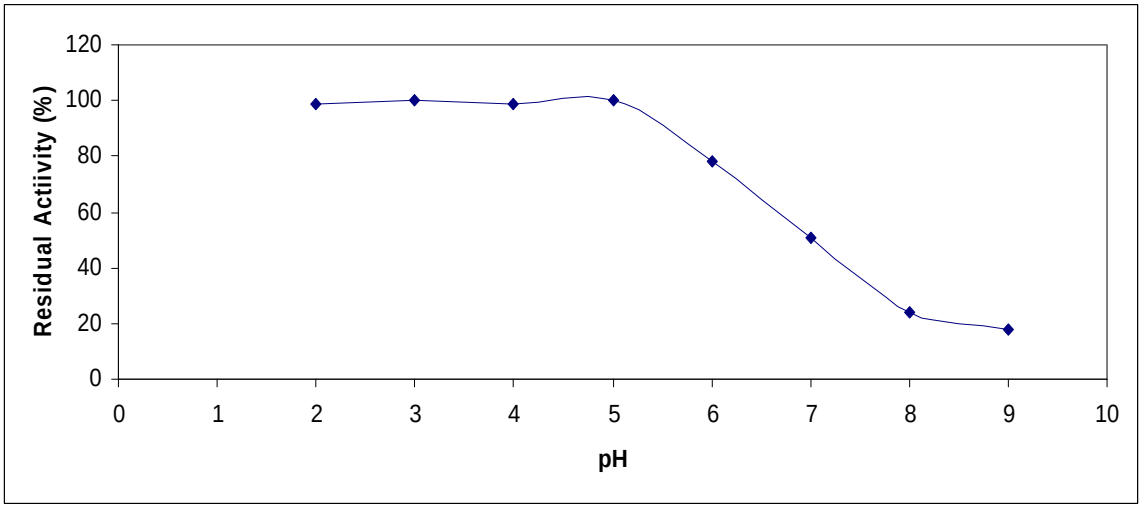


Figure.5 Effect of pH on phytase stability



Phytase isolated in the present study exhibits properties that make it a promising animal feed additive. Activity over a wide acidic pH range and stability up to 60°C are of particular significance. However, susceptibility to proteolytic cleavage has been observed. Purified phytases also find applications in agriculture as soil amendments and for improving plant nutrient availability (Sreedevi Sarsan, 2013; Gujar, Bhavsar and Khire, 2013). The enzyme analyzed in the present study has very good potential as a root soil additive or mineral supplement. Conversely, in order to assess the applicability in the true sense a complete purification of the enzyme is due. As many as 18 isolates tested positive for phytase production in the present study. Therefore mangrove rhizospheric soil may prove to be a powerful source of microbial phytases with desirable temperature, pH and stability attributes for industrial application.

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