Journal of Enzyme Engineering (2022)
Clausius Scientific Press, Canada

DOI: 10.23977/enzyme.2022.010102
ISSN 2616-2962 Vol. 1 Num. 1

Purification and Enzymatic Properties of β-galactosidase Produced from Lactobacillus acidophilus isolated from Dairy Waste-Water

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Keywords: *Lactobacillus acidophilus,* β-galactosidase, purification, specific activity

Abstract: β-Galactosidase producing *Lactobacillus* was isolated from the dairy industrial waste water in Rumuekini River State using standard microbiology (MRS), Biochemical and molecular techniques. Crude extract of β-Galactosidase was produced after successful screening of the isolates using mineral broth containing o-NPG through submerged fermentation system with optimized physiologic conditions. Four steps of purification was carried out: Ammonium sulphate, dialysis, ion exchange (DEAE-cellulose) and gel filtration (sephadex G-100). Crude extract was precipitated using 60% saturation of ammonium sulphate at pH 5.0 which gave the optimum precipitation of the protein with specific activity of 260.56 U/mg. Precipitation using ammonium sulphate carried out at pH 6.5 and 8.0 gave specific activity of 245 U/mg and 236 U/mg µmol/min of the precipitated protein respectively. The precipitates were further desalted through dialysis for twelve hours and specific activity of 425.17 U/mg was recorded afterwards. NaCl concentration gradient of 0.2-0.4M was found suitable in eluting the bound proteins from DEAE-cellulose resin with specific activity of 522.11 U/mg recorded from the pooled fractions. Further purification was done using sephadex G-100 and specific activity of 604.20 U/mg was recorded from the active pooled fractions. The purification table shows a 3.5 purification folds of β-galactosidase was gotten after ion exchange (DEAE-cellulose) and gel filtration (sephadex G-100) with enzyme percentage yield of 2.00%. The specific activity of β-galactosidase increased from 175.78 to 604.20 U/mg. Characterization of β-galactosidase gave optima pH and temperature of the enzyme at 5.0 and 70ºC respectively. Kinetic constants: K_M and V_{MAX} values obtained at various concentrations of p-NPG where 0.262 mM and V_{MAX} of 270.27µmol/min respectively. Ca^{2+} and Co^{2+}showed greater effect to β-galactosidase activity in a concentration dependent manner (0.03-0.05 M) when compared to Mn^{2+} and Fe^{2+}. The results from this study have shown that β-galactosidase producing *Lactobacillus acidophilus* has wide range of activity over physiologic conditions as regards to industrial and clinical standard operational procedures.

1. Introduction

β-galactosidase an enzyme of vast multiple applications is a 464-kDa homotetramer protein with 2,2,2-point symmetry (Jacobson *et al.,* 2014). The crystal structure of the enzyme was initially determined as monoclinic crystal form with the four peptides in the asymmetric unit (Matthews, 2015). Within each monomer, the 1023 amino acids form five well-defined structural domains which include: Domain 1 is a jelly-roll type barrel, Domain 2 and Domain 4 are fibronectin type III-like barrels**,** Domain 5, a β-sandwich and Domain 3 the central is a TIM-type barrel (Jacobson *et al.,* 2014). β-galactosidase is very specific for D-galactose and the two, three, and four galactosyl moiety positions are especially important for catalytic activity (McCarter *et al.,* 2012). The hydroxyls at those positions must each be present and in the correct orientation for the enzyme to catalyze the reaction (Loeffler *et al.,* 2009).

Studies have demonstrated that β-Galactosidases are widely found in microorganisms including those of bacteria, fungi, yeasts (*Acetomycetes*), plants especially in most diary leguminous plants such as jack beans (Chilaka *et al.,* 2002) others include almonds, peaches, apricots, apples and animal organs (Flood and Kondo, 2014; Haider and Husain, 2007a). *Aspergillus* sp. and *Kluyveromyces* sp. among the microbial domain are dominant producers of the enzyme for industrial purposes. β Galactosidase from *Kluyveromyces lactis* is one of the most widely utilized (Lee *et al*., 2013). β-galactosidases from bacterial sources (especially the Lactic acid bacteria LAB family) have been widely used during lactose hydrolysis because of the ease of fermentation of the disaccharide, high activity of the enzyme and good stability at physiologic ranges (Picard *et al*., 2005). However, galactosidases in β-D conformations from the fungi domain generally have acidic pH-optima in the range of 2.5–5.4, thus they are most effective and utilized during hydrolysis of lactose present in acidic products such as whey (cheese bye products) (Haider and Husain, 2007). *Kluyveromyces lactis* among all the yeast in the *Ascomycete* family is an important commercial source of β galactosidase because its natural habitat is the dairy environment (Kim *et al*., 2004; Tello-Solis *et al*., 2005). The production of β galactosidase by yeast could be of interest since this enzyme is used by the food industry for the production of lactose free milk, an outstanding industrial product used by a large number of lactose-intolerant people (Rech and Ayub, 2007). β-galactosidases are widely ubiquitous in the various tissues of plants (Chilaka *et al.,* 2002). These enzymes have been shown to be involved in a number of biological processes including plant growth (girthing), fruit ripening and in lactose hydrolysis.

Molecular approaches which show the role of β galactosidases in fruit development and ripening include their activity in bioactivation and stimulation of fruiting hormone the Abscisic acid (Li *et al.,* 2011).

Lactic acid bacteria (LAB) which constitute a diverse group of *Lactococci*, *Streptococci* and *Lactobacilli* have become a focus of scientific studies for the β-galactosidases production for three particular reasons: Lactose maldigesters (lactose intolerant individual) may consume some fermented dairy products with little or no adverse effects; Lactic acid bacteria (LAB) are generally regarded as safe (GRAS) so the enzyme produced from them might be used without extensive purification. Some strains have probiotic activity and as such improve digestion of lactose (Vasiljevic and Jelen, 2012). The present study looks into exhaustively the purification and enzymatic properties of β-galactosidases produced from *Lactobacilli acidophilus*.

2. Material and Methods

2.1 Materials

All the reagents/equipments used in this present study are of analytical grades and are calibrated at each use.

2.2 Methods

Sample Collection

Dairy industrial waste water was collected from effluent drainage surge tank of dairy industry located at Rumuekini front terminal, Portharcourt, Rivers state, Nigeria. The collected waste was taken to the laboratory in a clean sterile sample bottle for further analysis.

Isolation and Identification of Strains of *Lactobacilli* sp. from the Dairy Industry Waste Water.

Strains of the bacteria (*Lactobacilli* sp.) was isolated from the dairy waste water using standard microbiology (culturing/coliform counting, gram staining and microscopy mounting) and biochemical (sugar fermentations, nitrogen digestions) technique as described by Ezeonu *et al.*(2013). Colony forming units counts (CFU/ml) was calculated using the formular:

TOTAL HETEROTROPHIC COUNTS X RECIPROCAL OF VOL.OF INOCULUM
X RECIPROCAL OF DILUTION FACTOR

Molecular Identification of *Lactobacilli* sp.

Genomic DNA (gDNA) from the selected isolate was obtained using the QIA amp DNA Mini Kit. The 16S rDNA gene was amplified by RT-PCR using the forward (5'-AGTTTGATCATGGTCAG-3') and reverse (5'-GGTTACCTTGTTACGACT-3') primers. The amplified DNA sequence was compared to the Gen Bank database of National Center for Biotechnology Information (NCBI) using the BLAST program (Kumar *et al.*, 2016).

Screening of *Lactobacilli* sp. for β-galactosidase Production

Identified *Lactobacilli* sp. was screened for β-galactosidase producing ability using Demanragoshie sharpie broth supplemented with 2mM p-NPG as described by Gheytanchi *et al.*(2010). The inoculated culture broth was incubated at 37°C for 3 days.

Assay β-galactosidase activity

β-galactosidase was assayed according to the method described by Chilaka *et al.* (2002) using p-NPG as the substrate. Assay mixture contained 0.5 ml of enzyme solution, 0.1 ml of 2mM p-NPG and 0.5 ml (0.1M) phosphate buffer solution (pH 6.5). The mixture was incubated at 50°C for 30 min. The reaction was stopped using 4ml of 0.1M NaOH and absorbance was taken at 400 nm. One unit of β-galactosidase activity is defined as the amount of p-Nitrophenol (µmole) given off per minute from p-Nitrophenyl β-D- galactopyranoside by β-galactosidase.

Protein determination

The protein content of the enzyme was estimated as described by Lowry *et al.* (1951) using bovine serum albumin (BSA) as the standard protein. Absorbance was taken at 750 nm.

Enzyme production.

Submerged fermentation technique was used for β-galactosidase production as described by Allam *et al*. (2016). Flasks (250 ml) containing 100 ml of liquid media optimized for β-galactosidase production contained: 1% peptone, 0.4 % K_2HPO_4, 1 % Lactose, 0.2ml tween 80, 0.01% sodium acetate, 01 % di-ammonium citrate, 0.05 % $MgSO_4.7H_2O$ and 0.2% $MnSO_4.4H_2O$. The medium were sterilized at 121°C/ 15psi for 20 min using the electronic autoclave. The enriched broth was cooled and inoculated with 2ml of the microbial suspension using the syringe and incubated at pH 6.0 and at 37 °C for 12 days. Fermentation medium was filtrated using a muslin cloth of pore size 2mM. The filtrate (crude extracts) was assayed for β-galactosidase activity.

Purification of β- galactosidase Produced from *Lactobacilli acidophilus.*

Ammonium Sulphate Precipitation Profiling

This was carried out as described by Allam *et al*. (2016). Test tubes numbering eight were used to form the ammonium sulphate precipitation profile. β- galactosidase were precipitated by gentle stirring at 20-90 % saturation of solid ammonium sulphate at intervals of 10 % in each test tube. The test tubes containing the enzyme solutions with the ammonium salts were filtered using the whattman's filter paper. Precipitates from the individual percentage ammonium sulphalte saturations collected from the filter paper were redissolved respectively in five (5 ml) of 0.1M sodium phosphate buffer pH 6.0. β-galactosidase activity of the precipitates and the supernatants were assayed simultaneously as described above to determine the percentage ammonium sulphate saturation that precipitated the enzyme from the solution. Also, enzyme solution (10 ml) prior to precipitations with ammonium sulphate was adjusted to pH values of five (5) and eight (8) using 1% HCl v/v and 0.1M NaOHaq respectively.

Ammonium sulphate precipitations were carried out at the adjusted pH values (5 and 8) of the crude enzyme solutions as described at the beginning of this section.
Precipitated extracts gotten after separation with No 1whattman's filter paper from the respective percentage (%) ammonium sulphalte saturations were redissolved respectively in five (5 ml) of 0.1 M sodium phosphate buffer pH 6.0. β- galactosidase activities of the precipitates and the supernatants were assayed simultaneously.

Dialysis of the Precipitated Crude Extracts

Dialysis of the precipitated crude protein across gradients was carried out as described by Chen *et al*. (2008). Redissolved precipitated extracts were dialyzed against precipitation pH values and salt concentration gradients (0.01 M) created across the dialysis bag for twelve (12 hours) in a cold ice pack container. The concentration of the buffers used to create concentration gradients was maintained throughout the dialysis time while the ice packs were continually changed when the packs defrosted.

Ion Exchange Chromatography

This was carried out as described by Chilaka *et al*. (2002). Positively charged ionic resin, diethyl amino ethyl-cellulose (DEAE- cellulose) was used as the stationary phase for the chromatographic purification. Sodium phosphate (0.01 M) of pH 7.5 was used as the elution buffer. NaCl (0.01-1M) was prepared in 100 ml of the buffer solution (pH 7.5) to create various ionic strengths salt gradients. Bound proteins was washed discontinuously with each of the salt

concentrations prepared while ten fraction tubes (5ml marked) carefully labeled for each salt treatments was collected at each elution. Unbound proteins were washed off with the 0.01 M phosphate buffer (pH 7.5). Enzyme activity was determined in each eluted fraction tubes as described above.

Gel Filtrations

This was carried out as described by Allam *et al.* (2016). Sephadex G-100 used as the stationary phase for the purification was gently packed in the column. The packed gel was equilibrated with 0.1M sodium phosphate buffer solution (pH 6.0) before loading the enzyme solution. A total of 70 fractions were collected using 5ml fraction tubes at a flow rate of 5ml per approximate 15 minutes. The protein concentration of each fraction was determined using a spectrophotometer at wavelength of 280 nm. β-galactosidase activity of each fraction was also assayed spectrophotometrically at 400 nm as described above.

Studies on Partially Purified Enzyme

Effect of pH on β-galactosidase Activity

The optimum pH for β-galactosidase activity was determined using 0.1M sodium acetate buffer of pH ranging from 3.5 - 5.5, sodium phosphate buffer of pH 6.0 - 7.0 and Tris-HCl buffer of pH 7.5 - 9.0 at intervals of 0.5 units as described by Chen *et al.* (2008). Partially purified β-galactosidase were dispensed in the various prepared buffers, β-galactosidase activity was determined as described above.

Effect of Temperature on β-galactosidase Activity

This was carried out as described by Chen *et al*. (2008). The optimum temperature was determined by incubating the enzyme with its substrate solution (p-NPG) at temperatures ranging from 30-70°C using a thermo regulator (water bath) at interval of 10°C for 30 minutes and at its optimum pH. β-galactosidase activity was assayed as described above.

Effect of Substrate Concentration on β-galactosidase Activity

This was carried out as described by Chilaka *et al*. (2008). Effect of substrate concentrations on the activity of β-galactosidase was determined by incubating the enzyme with 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 mM of p-NPG solutions at the optimal pH and temperature recorded in the previous studies. The V_{Max} and K_M values of the enzyme were determined using the double reciprocal plot. Activity of β-galactosidase was determined as described in above.

Effects of Divalent Metal Ions on the Activity of β-galactosidase

This was carried out as described by Li *et al.* (2011). Chloride salts of different divalent metals: Calcium (Ca), Manganese (Mn), Magnesium (Mg) and Cobalt (Co) of concentration 30- 50 mM respectively were incubated with the enzyme at their optimal pH and temperature; activity of the enzyme at each treatment with the metal salts was determined as described above.

3. Results

Microbial isolations and molecular identifications.

Effluent surge tank of the dairy industry located at Rumuekini, Rivers state showed high heterotrophic microbial diversity and activity. Plate one below shows the visible colonies of strains

of *Lactobacilli* sp. on DeManRagoshie Sharpie medium (MRS) isolated from the dairy waste water; total heterotrophic counts of 2.8 x 10^5Cfu/ml of the bacteria isolate were identified on the plate.

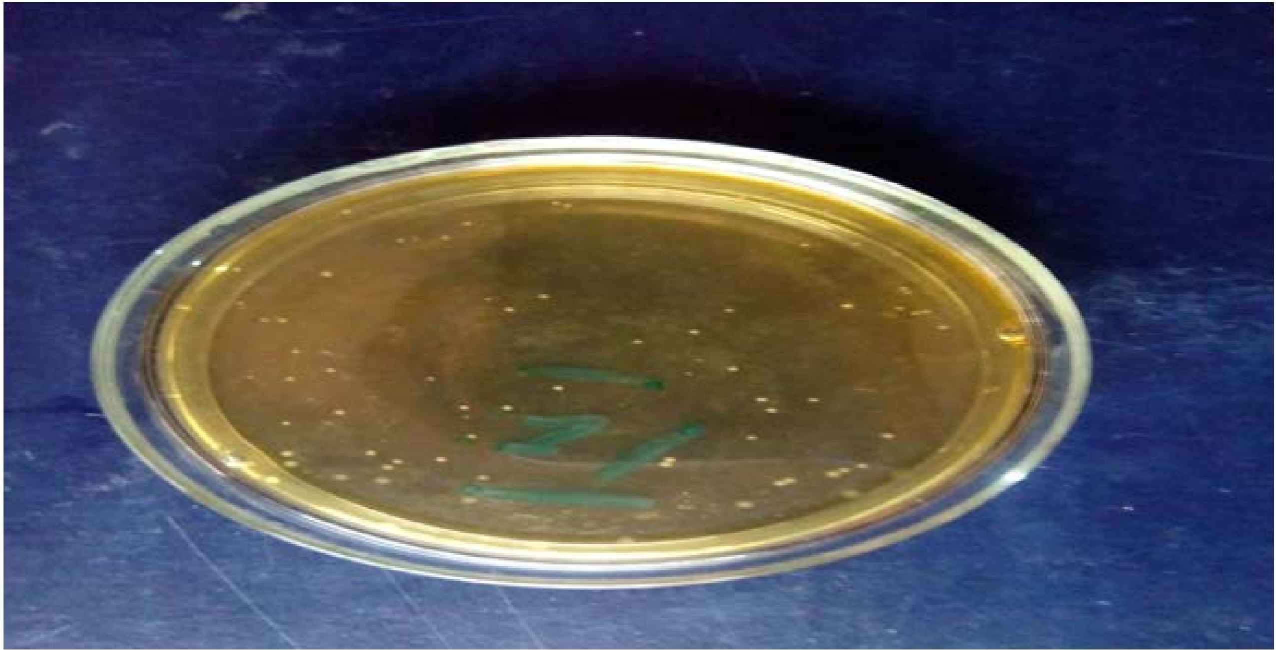

Plate 1. Pure colonies of strains of Lactobacillus sp. on MRS medium (2.8 x 10^5Cfu/ml)

Plate 2 below shows the micrograph of the bacteria suspension under an objective magnification of x100; the micrograph shows clusters of rod shaped bacteria cells with stained background using safranine dye.

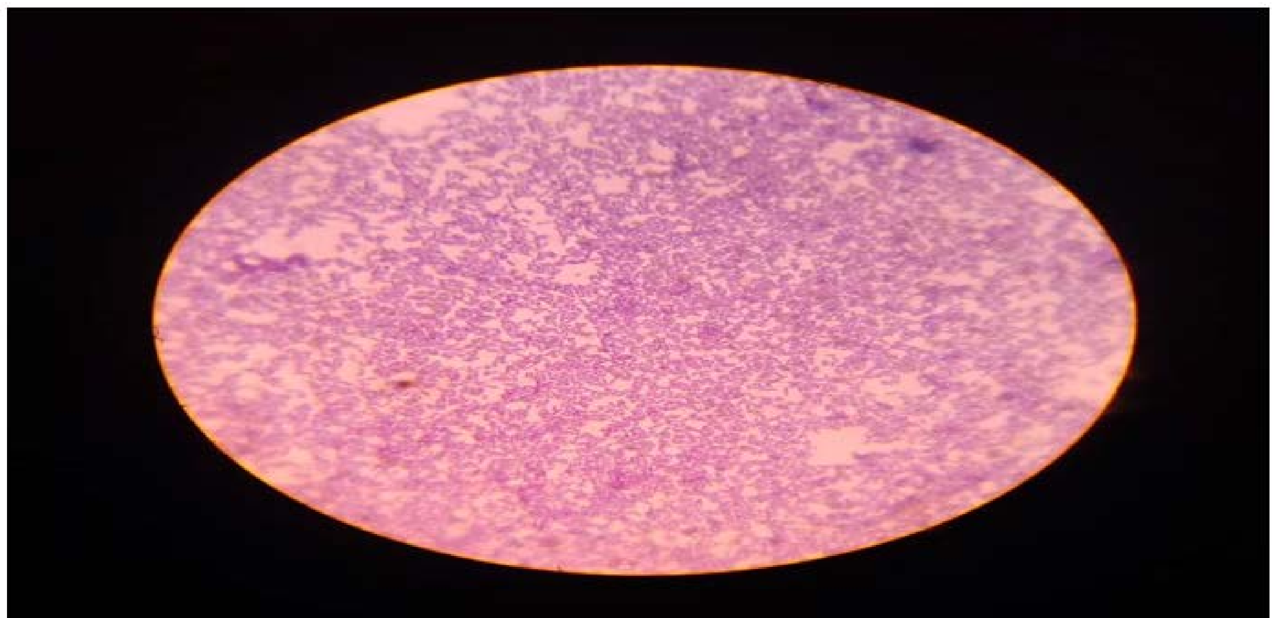

Plate 2. Micrograph of mount strains of Lactobacilli sp. under the objectives of light microscope of x100 magnification.

Figure 1 below shows the phylogentic relatedness tress of the identified *Lactobacillus acidophilus* after the genomic sequencing with ascribed NCBI accession number of JX255677

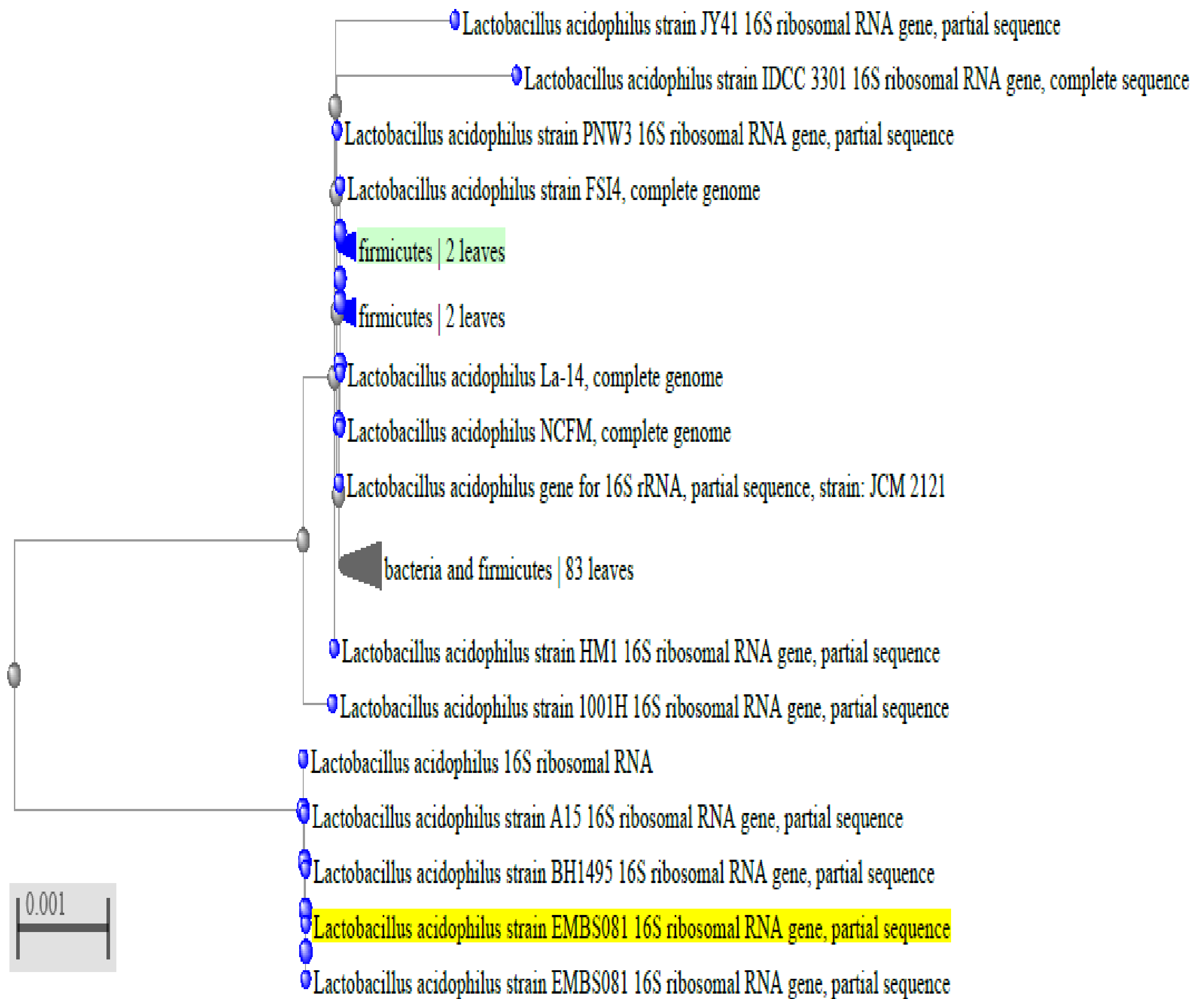

Figure 1. Phylogenic evolutionary relatedness of strains of Lactobacilli sp. obtained using NCBI BLAST tools.

Screening of the Isolates for β-galactosidase Production

Figure 2 below show the yellow colouration of p-NPG substrate used for screening of *Lactobacilli acidophilus* for β-galactosidase production in mineral broth medium.

Fig 2. Chromogenic detection of Lactobacilli sp. with potentials of β- galactosidase production using p-NPG substrate.

Effect of Incubation Time on β- Galactosidase Production

Studies on the effect of incubation time on the production of β-galactosidase from *Lactobacilli acidophilus* in a submerged fermentation system showed that the highest activity and protein concentration were obtained on day 12 (126µmol/min) and day 13 (0.72 mg/ml), respectively of incubation (figure 3).

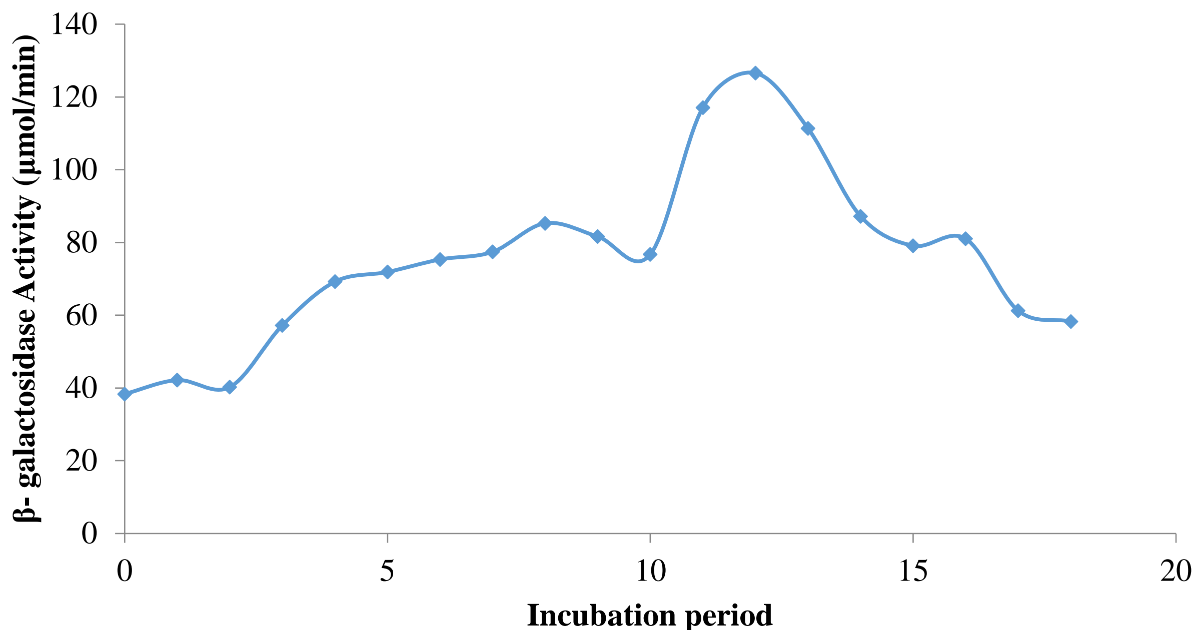

Fig 3 Effect of incubation period on β-galactosidase production

Ammonium Sulphate Precipitations

Fig. 4, 5 and 6 showed the precipitation profile of the crude extracts from the solution using ammonium sulphate of various percentages forming the precipitation profile. Crude extracts were precipitated at various physiologic pH ranges of 5.0, 6.0 and 8.0. Figure 4 show the protein precipitation at pH 5.0 with maximum protein precipitation at 60% saturation of the ammonium salt, figure 5 show the precipitation of the crude extracts at pH 6.0 with maximum protein precipitation at 70 % saturation of the ammonium salt while figure 6 show the precipitation of the crude extracts at pH 8.0 with maximum protein precipitation at 80 % saturation of the ammonium salt.

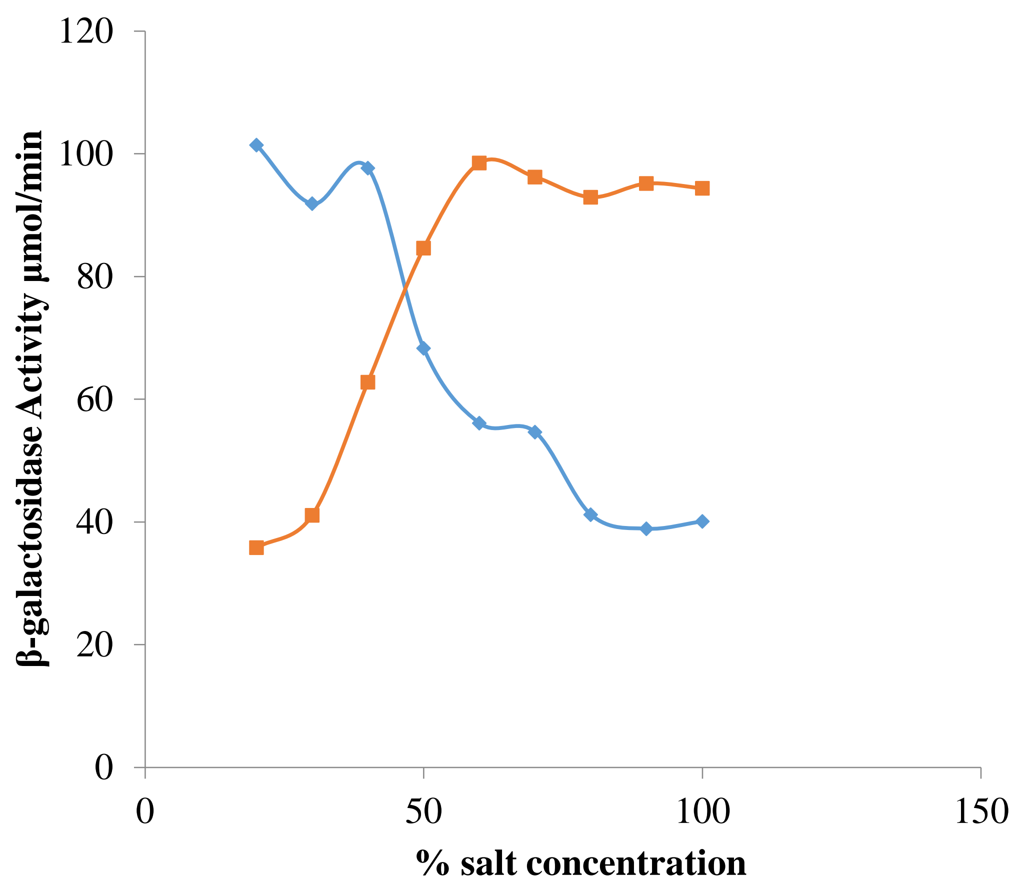

Fig. 4 Ammonium sulphate precipitation profile of crude extracts of β –galactosidase from Lactobacilli acidophilus at pH 5.0.

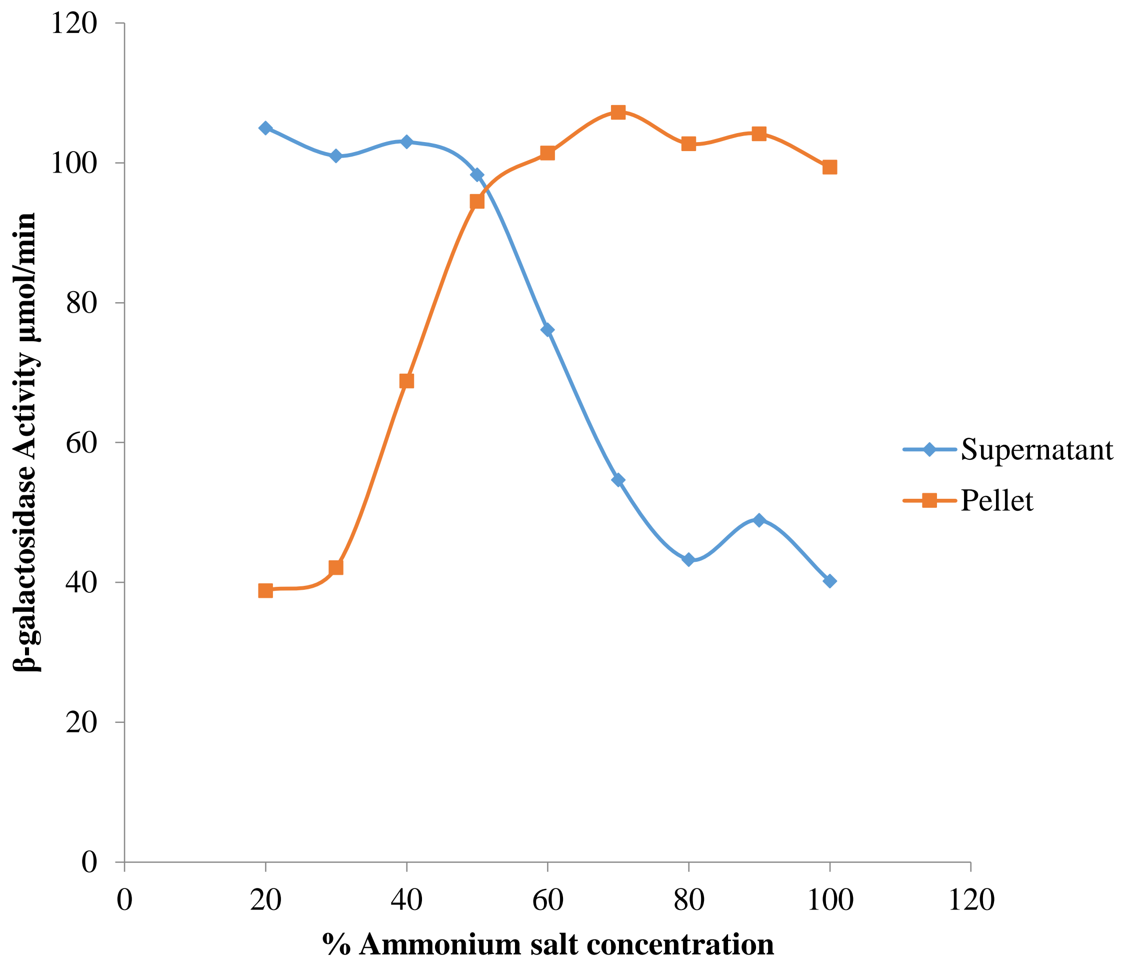

Fig. 5 Ammonium sulphate precipitation profile of crude extracts of β –galactosidase from Lactobacilli acidophilus at pH 6.0.

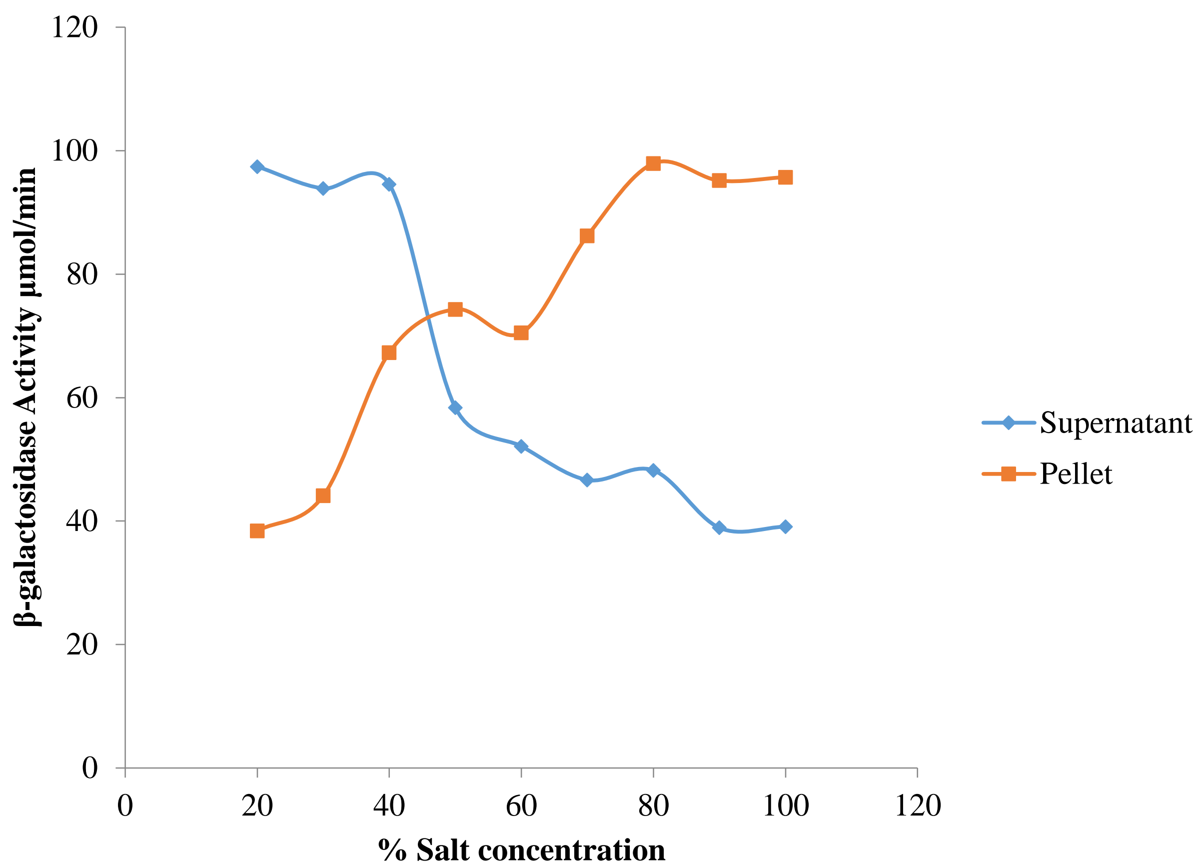

Fig. 6 Ammonium sulphate precipitation profile of crude extracts of β –galactosidase from Lactobacilli acidophilus at pH 8.0.

Ion Exchange Chromatography Separation of the Desalted Protein

Fig. 7 shows the ion exchange elution profile of the desalted protein; ion exchange separation resin Diethylaminoethyl-cellulose (DEAE-cellulose) binds to the protein at pH 7.5. Sodium chloride (NaCl) elution solution of concentration 0.3-0.5 M was able to desorbed the bound protein from the anionic exchanger resin after discountinous gradient washing of the column with the prepared salt concentrations.

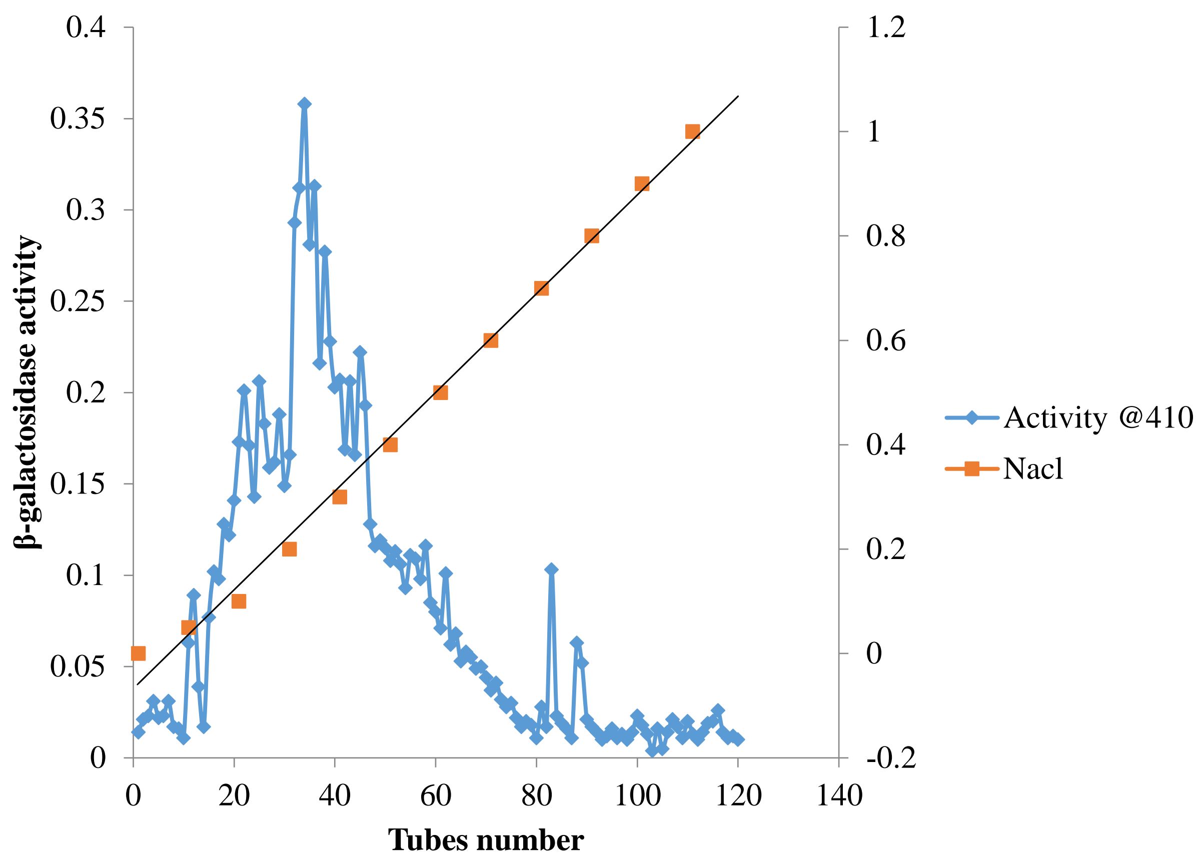

Fig. 7 DEAE elution profile of β –galactosidase produced from Lactobacilli acidophilus in a submerged fermentation system.

Gel Chromatography Purification of the Protein

Fig. 8 shows the gel elution profile of the proteins using sephadex G-100 of bed height 75 and column volume of 235.65cm^3; from the figure, proteins elution fractions from the column start with tube number 14-22 and then from starting from tube number 29, 30, 31 and 36 fractions. Void volume (Vo) of the elution profile was recorded from tube number 0-13.

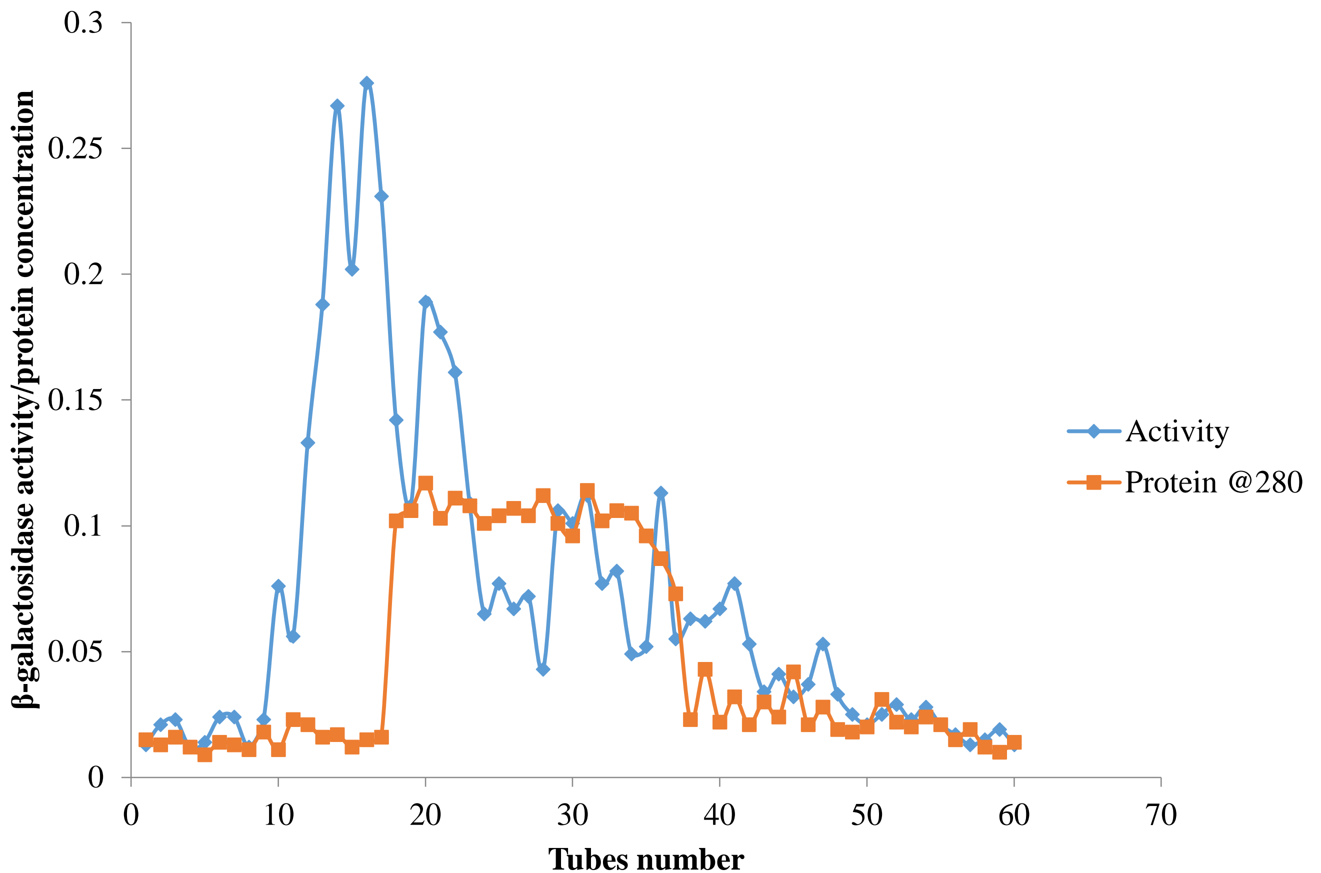

Fig. 8 Gel elution profile of β–galactosidase produced from Lactobacilli acidophilus in a submerged fermentation system.

Purification Table of the Protein

Table 1 below shows the purification profile of β-galactosidase from *Lactobacillus acidophilus,* from the table, four steps of purification were carried out on the crude extracts: ammonium sulphate, dialysis, ion exchange (DEAE-cellulose) and gel filtration (sephadex G-100). Crude protein extract was purified upto 3.5 folds after ion exchange (DEAE-cellulose) and gel filtration (sephadex G-100) with percentage yield of 2.00%. The specific activity of β-galactosidase increased from 175.78 to 604.20 U/mg after gel filtration.

Table 1 Purification Table of the Protein

	Volume (ml)	Protein (Mg)	Total protein	Activity µmol/min	Total activity U/ml	Specific activity U/mg	Purification folds	Percentage yield

Crude enzyme	1000	0.72	720	126.56	126560	175.78	1	100
Ammonium sulphate precipitation	250	0.378	94.5	98.49	24622.5	260.56	1.48	19.45
Dialysis	100	0.201	20.1	87.46	8746	425.17	2.42	6.85
Ion exchange (DEAE cellulose resin	50	0.152	7.60	79.36	3968	522.11	3.00	3.2
Gel filtration (G-100)	30	0.110	3.30	66.46	1993.80	604.20	3.50	2.00

Characterization of β-galactosidase

Optimum pH Determination

Fig 9 below shows the effect of pH on β-galactosidase activity; from the figure, the enzyme showed optimal activity at pH 5.0 with peak activity of 77.62µmol/min. β-galactosidase activity decreases significantly from pH 7-8.5.

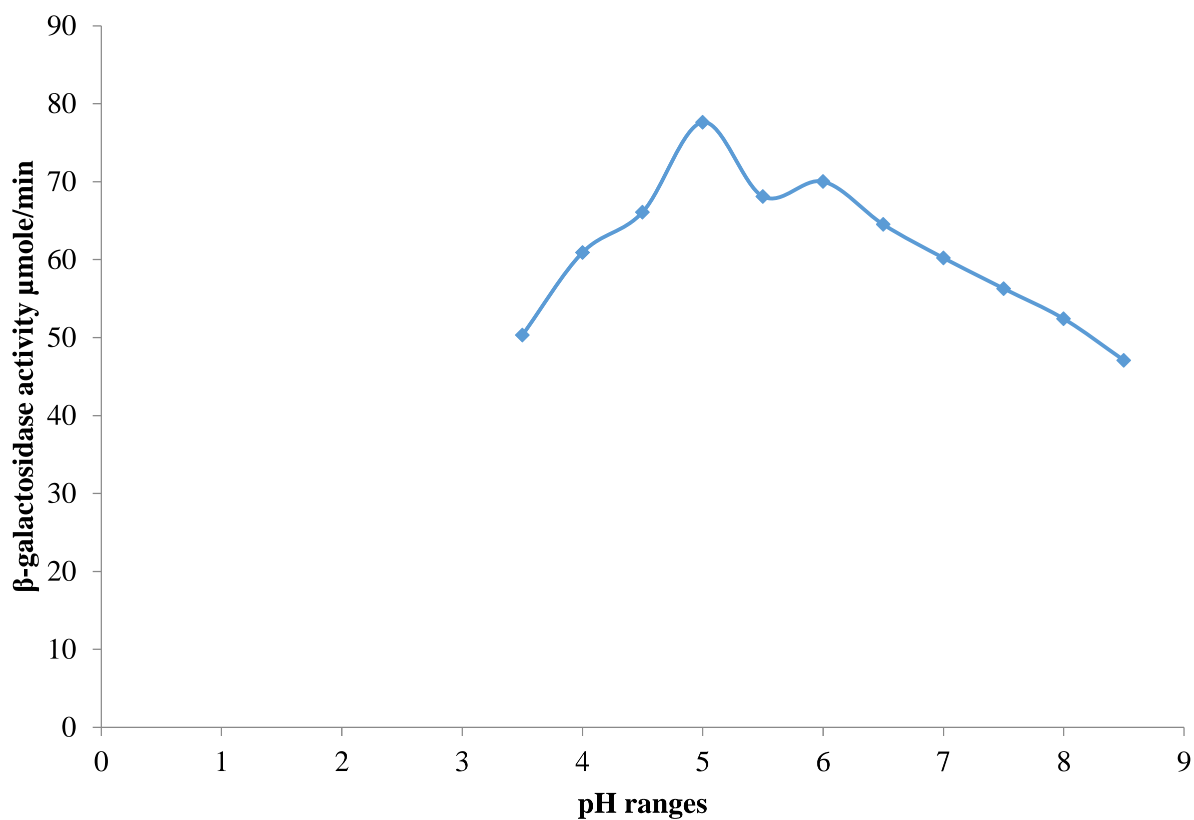

Fig 9 Effect of pH on β-galactosidase activity.

Optimum Temperature Determination

Fig 10 below shows the effect of temperature on β-galactosidase activity; from the figure, the enzyme showed optimal activity at 70°C with peak activity of 78.21 µmol/min. β-galactosidase showed an ambient increase in activity at 50°C. Sharp decrease in activity was seen at 60°C and from 80-90°C respectively.

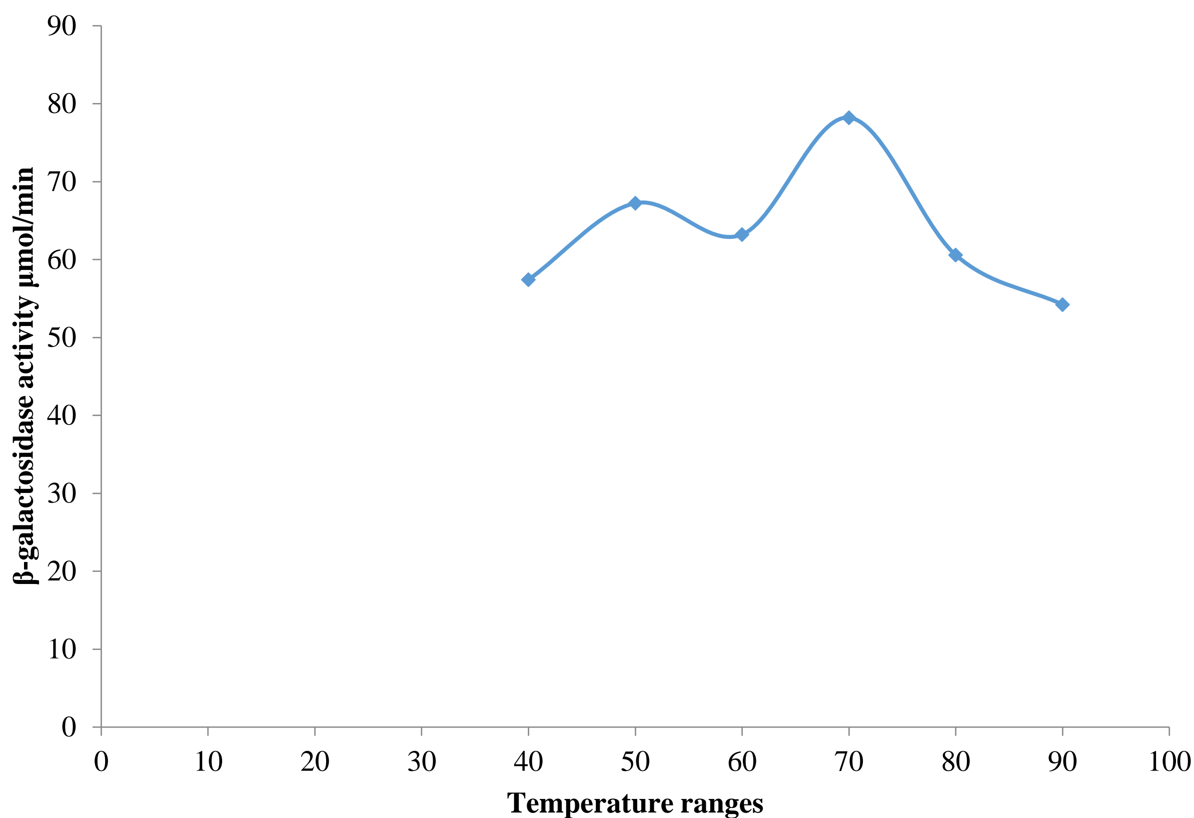

Fig. 10 Temperature activity profile of β –galactosidase produced from Lactobacilli acidophilus in a submerged fermentation system

Determination of kinetic Constants (K_M and V_{max})

Figure 11 and 12 show the Michealis-Menten and Lineweaver-burk reciprocal plots of β-galactosidase activity at different concentrations of p-NPG respectively. Michaelis-Menten constant (K_M) and maximal velocity of catalysis (V_{Max}) of 0.262 mM and 270.27µmol/min were obtained at different concentrations of p-NPG.

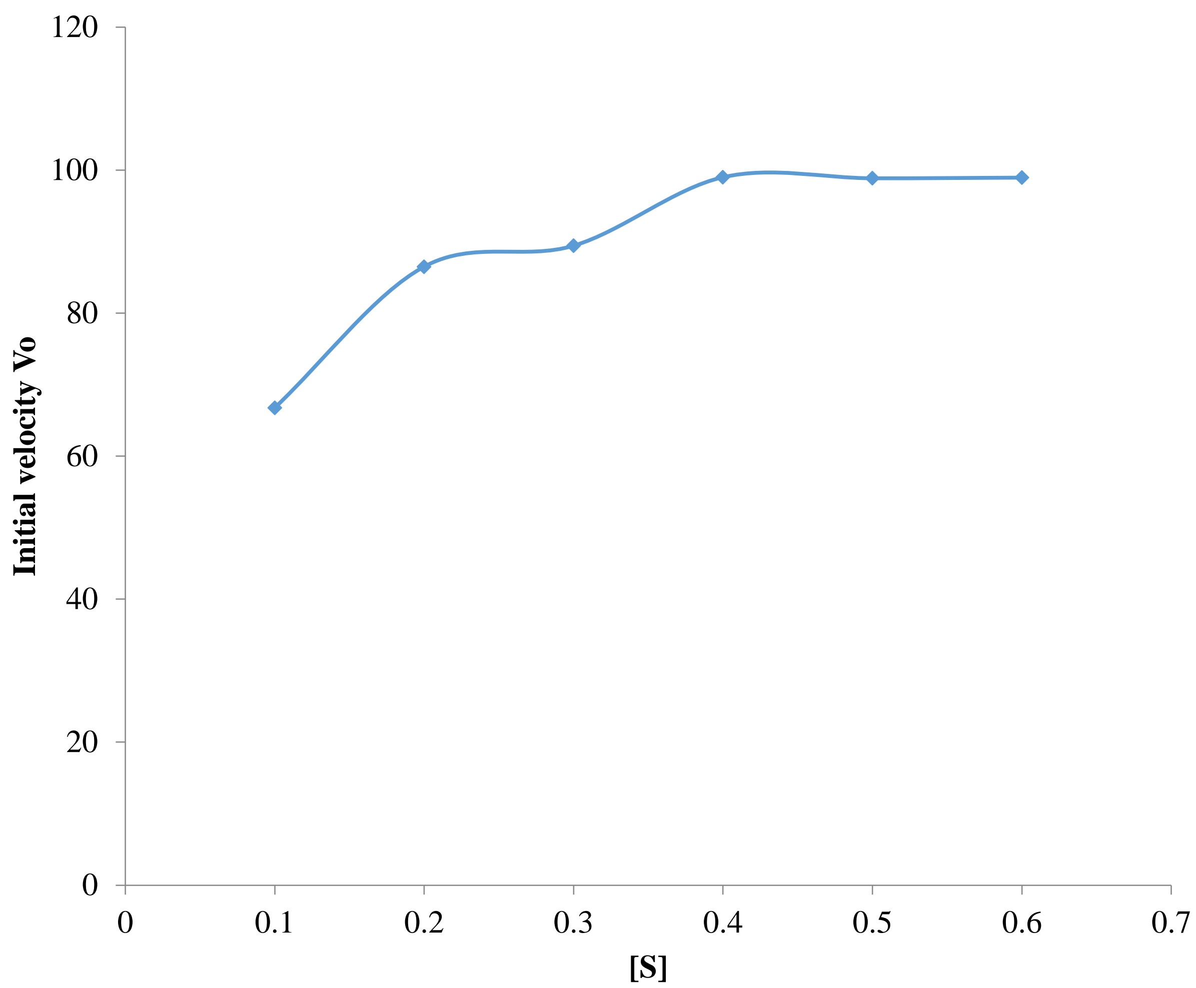

Fig. 11 Michealis-Menten plot of β –galactosidase activity at various concentrations of p-NPG

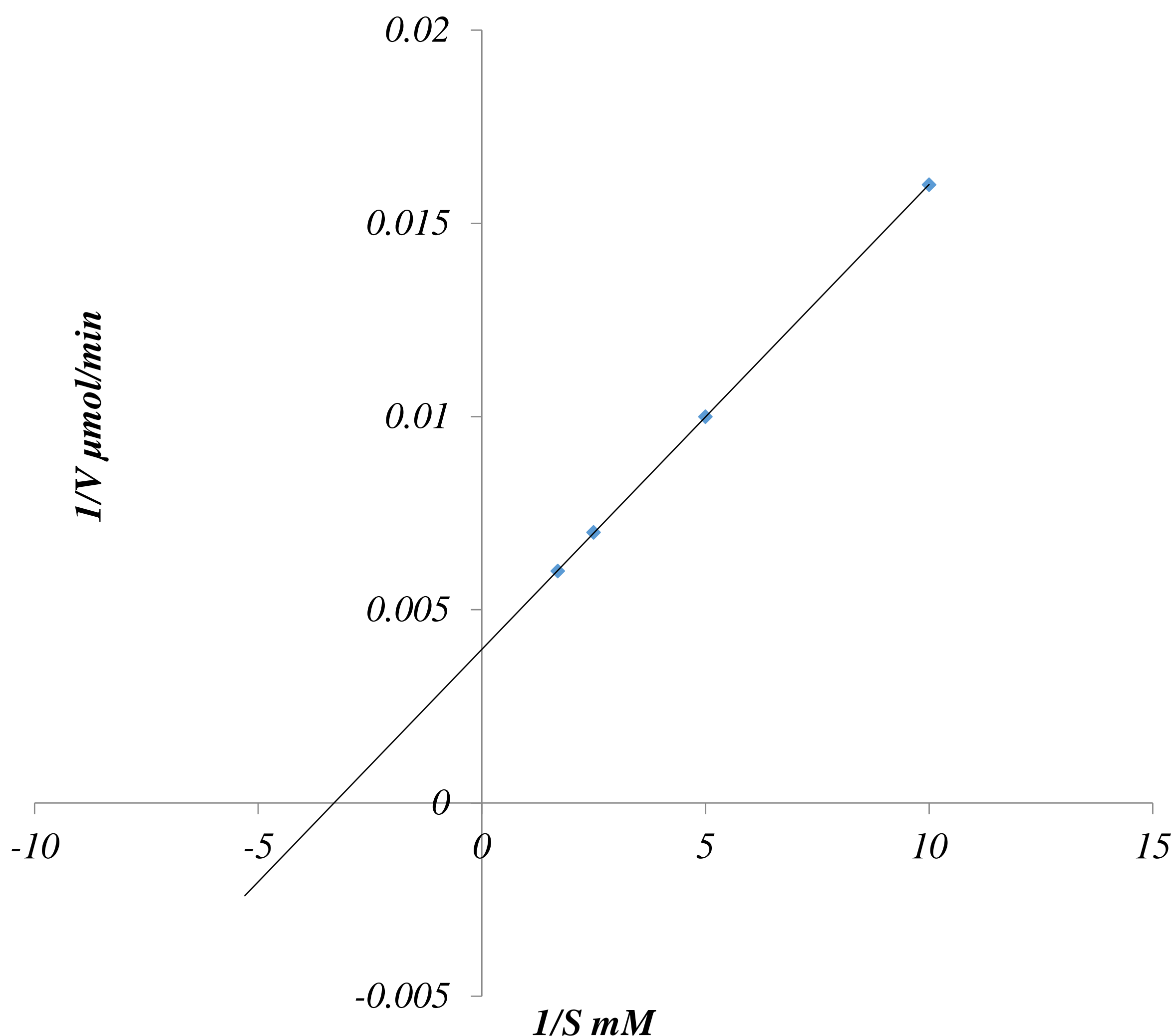

Fig. 12 Lineweaver-burke reciprocal plot of β –galactosidase activity at various concentrations of p-NPG.

Effect of Divalent Metals on β-galactosidase Activity

Figure 13 shows the effect of selected divalent metal ions on the enzyme activity; from the figure, divalent metal ions of Ca^{2+}, Mn^{2+}, Fe^{3+} and Co^{2+}were positive effectors to the enzyme activity relative to the control experiment (metal ions blank). Metal ions of Ca^{2+} and Co^{2+}showed greater effect to the enzyme activity when compared with the control than Mn^{2+} while Fe^{3+} showed the least effect to the activity of the enzyme in a concentration dependent rate (0.03-0.05M).

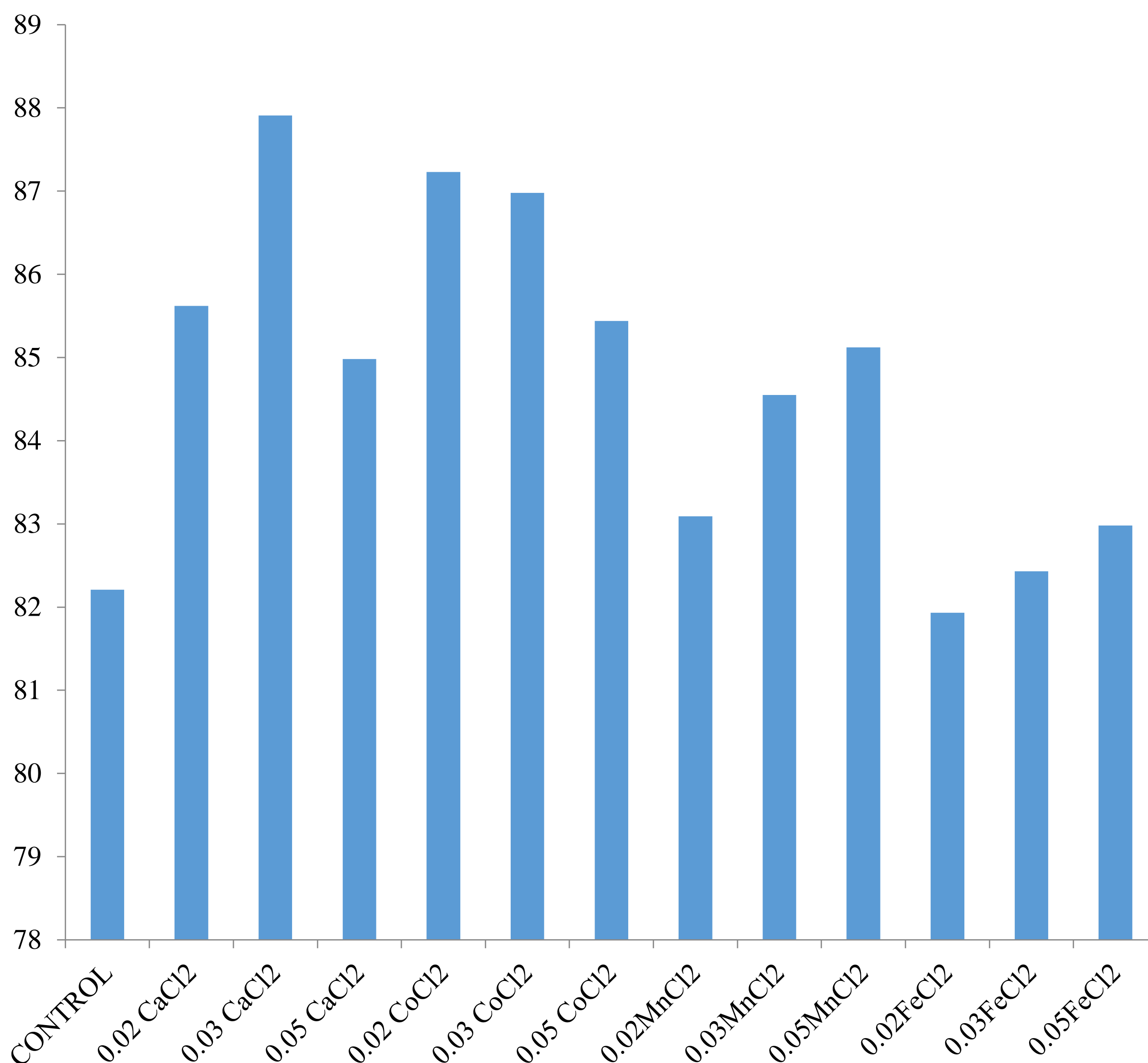

Fig. 13 Effect of divalent metal ions (chloride of the salts) on β –galactosidase produced from Lactobacilli acidophilus in a submerged fermentation system.

4. Discussion

Molecular test (16S rDNA) was used to identify the pure isolates of *Lactobacilli*. Electrophoretogram of the amplified genome of *Lactobacilli* using RT-PCR showed a typical band at 750 bp. Kumar *et al.* (2016) reported that band size of 650-800 are typical of bacteria. *Lactobacillus acidophilus was* identified after the genomic sequencing with ascribed NCBI accession number of JX255677.

Strains of *Lactobacilli acidophilus* isolated from the dairy effluent showed the ability to produce β-galactosidase when screened with p-NPG as shown in figure 2. Formation of yellow colouration in the nutrient broth after 48 hours of incubation at 37°C suggests a positive test for β-galactosidase production. Gheytanchi *et al*. (2010) reported similar observation, working with *Lactobacillus* from milk and cheese using o-NPG as their standard screening substrate.

Protein precipitation from the crude extracts solution using ammonium sulphate was carried out at three different pHs: 5.0, 6.5 and 8.0. The variation of pH during this process is to corroborate the relationship between protein precipitation and their isoelectric points (PI). It is noteworthy that at every isoelectric point of molecules in a solution, their solubility decreases greatly or becomes zero. Isoelelctric point of proteins is that pH at which every protein assumes a zwitteronic charge (net charge of zero) (Nguyen *et al*., 2006).

Studies on the effect of incubation period on the production of β-galactosidase from *Lactobacilli acidophilus* showed that the highest β-galactosidase activity and protein concentration were obtained on the 12th and 13th day of fermentation, respectively. Extracellular protein production at these observed days (12 and 13) respectively is evident of catabolite inducement of the substrate present in the fermentation media to the organisms for higher protein production (Allam *et al*., 2013). *Lactobacilli* a starter culture bacterium for the enzyme production is a known probiotic in food industries, they are known to be fastidious in nutrient requirements and growth and as such have relatively large lag period with their prebiotic before switching on their clusters genes (*Lac-operon*) (Cohn and Monod, 1951).

In this present study, at pH 5.0, 60% ammonium sulphate saturation was found suitable to precipitate protein with highest β-galactosidase activity (98.49 μmol/min) whereas at pH 6.0 and 8.0, 70% and 80% of the salt saturations were able to precipitate protein with highest β-galactosidase activity respectively. pH 5.0 and 60% saturation of ammonium sulphate gave the best precipitation of the protein. Nguyen *et al*. (2006) reported an optimal precipitation of their protein produced from *L. reuteri* at 60% saturation of ammonium sulpahte and pH 6.0. Oluwaniyi *et al* (2016) reported the precipitation of β–galactosidase from *Kluveroyces lactis* isolated from a yoghurt waste site at 65%% saturation of ammonium sulphate and at pH 7.0.

The precipitated protein was de-salted for 12 hours using 2mm dialysis bag in an ice pack container. After the process, 20 ml of desalted protein was recovered after initial 10 ml was introduced into the bag with increased specific activity of 425.17 U/mg.

Two peaks of enzyme activity was observed after discontinuous washing with 0.0-1M gradient of NaCl solutions with flow rate of 5 ml/10 min. Active fractions of the protein was observed at elutions of 0.3-0.5 M of NaCl solution; however the active fractions were pooled together with specific activity of 522.11 U/mg. The protein was purified upto 3.00 folds and percentage yield of 3.2% was recovered. Chilaka *et al* (2002) reported single peak of β-galactosidase activity from Jack bean after ion exchange using DEAE-cellulose resin with purification fold of 2.16 and percentage yield of 53.52%. Oluwaniyi *et al*. (2013) reported double peaks of enzyme activity and protein concentration of β–galactosidase produced from *Kluveromyces lactis* after ion exchange chromatography using DEAE-cellulose resin.

Sephadex G-100 was used for further purification of the protein to various molecular sizes and weight. Elution was done with 0.1M of sodium phosphate buffer at pH 6.0. Protein flow rate of 5ml/18min was observed. Double peaks of enzyme activity and protein concentrations were seen from fraction tubes number 14-22 and then from starting from tube number 29, 30, 31 and 36 fractions. As reported by Chilaka *et al*. (2002), they stated that there appears to be a relationship between dialysis of enzymes usually after ammonium sulpahte precipitation and the presence of isoenzymes., they went further to state that when dialysis is replaced by gel filtration in enzyme purification, isoenzymes were lost out. Multiple peaks of the enzyme activity could be attributed to ionic scrambling encouraged by dialysis and this leads to formation of aggregates with incorrect

ionic bond pairs. Such aggregates express ionic heterogeneity on any column chromatography (ion exchange and size exclusion) (Chilaka *et al.,* 2002). β-galactosidase was purified upto 3.5 folds after the gel filtration (sephadex G-100) with percentage yield of 2.00%.

The specific activity of the enzyme increased from 175.78 to 604.20 U/mg after gel filtration. Akolkar *et al*. (2004) reported specific activity of 568.61 U/mg and purification folds of 21.2 after gel filtration of β-galactosidase produced from *Lactobacillus acidophilus* isolated from fermented ragi.

Optimum pH for β-galactosidase produced from *Lactobacillus acidophilus* was 5.0. Oluwaniyi reported optima pH of 7.5 and 6.5 for galactosidase produced from *Kluveromyces lactis* using o-NPG and lactose substrates respectively while Nguyen et al. (2006) reported an optimum pH for β-galactosidase produced from *Lactobacillus reuteri* at narrow acidic pH range of 4-6. Adalberto *et al*. (2010) reported an optimum pH for β-galactosidase produced from *Kluyveromyce lactis* at pH 7.5.

Optimum temperature for β-galactosidase produced from *Lactobacillus acidophilus* was at 70°C. Optimum activity of β-galactosidase at this high temperature maybe attributed to the nature of the organism used for the enzyme production. Strains of *Bacilli* generally are ubiquitous rod shaped bacteria found in most harsh environment (heavily polluted sites, thermal inflicted region). They are mostly used in food processing and other industrial biased applications where high temperature is needed (Vallero, 2010).

Kinetic constants (K_M and V_{MAX}) of β-galactosidase determined during the study showed K_M of 0.262 mM and 3.65 mg/ml for p-NPG. Oluwaniyi reported K_M of 3.52mM and 18.2 mg/ml for o-NPG and lactose respectively on β-galactosidase produced from *Klyuveromyce lactis.*

Metal ions generally play important roles in the biological function of many enzymes (both clinical and industrial implicated ones) (Riordan, 1977). Divalent metal ions (double cationic charged) are mostly implicated in enzyme active sites where they are directly involved in catalysis, maintenance of structural compatibility of the enzyme or in affinity binding of enzyme substrates together with the arrays of amino acids making up the protein (Adalberto *et al.,* 2010). Divalent metals of: calcium, cobalt, manganese and iron with concentrations of 0.03-0.05M showed positive effect on β-galactosidase activity in the presence of the control experiment. The activation was concentration dependent for all the divalent metals; however, calcium and cobalt ions showed greater effect on β-galactosidase activity relative to the control that Manganese ion while Iron II ion showed the least effect on the enzyme activity. It was seen that 0.03 M of Ca^{2+} (87.91µmol/min) and Co^{2+} (86.98µmol/min) gave the best effect to β-galactosidase activity relative to the control (82.21µmol/min), however it was noticed that at 0.05M of both metals (calcium and cobalt) the effect retards. Manganese and Iron ions gave peak effect on β-galactosidase at 0.05M concentration respectively.

5. Conclusions

Every biological based industry (especially the food industry) today relies on the use of easily accessible and cheap biocatalysts. β-galactosidase is a multi-purpose enzyme that has find its way in many producing industries including those of foods, clinical and environmental based research industries. The present study has shown the basic enzymology and kinetic properties of β-

galactosidase produced from *Lactobacillus acidophilus* isolated from an effluent site. β-galactosidase exhibited stable enzymatic properties over a wide range of physiologic conditions (pH and temperature) and huge affinity to its substrate shown in its K_M and V_{max} values respectively.

References

*[1] Adalberto, P., Massabni, A., Carmona, E., Goulart, A., Marques, D. and Monti, R. (2010). Effect of divalent metal ions on the activity and stability of β-galactosidase isolated from Kluyveromyces lactis. Journal of Basic and Applied Pharmaceuticular Sciences, **31**(3): 143-150.*

[2] Allam, R., Aly, M., El-zhrany, K. and Shafei, M. (2016). Production of β-Galactosidase enzyme from Lactobacillus sp. RK isolated from different sources of milk and dairy products. International Journal of ChemTech Research, 9 (10): 218-231.

[3] Chen, W., Chen, H., Xia, Y., Zhao, J., Tian, F. and Zhang, H. (2008). Production, purification and characterization of a potential thermostable galactosidase for milk lactose hydrolysis from Bacillus stearothermophilus. Journal of Dairy Science, 91:1751–1758.

[4] Chilaka, F.,Nwachukwu, A. andUvere, P.(2002). Thermal stability studies of β –galactosidase from germinating seeds of the brown beans, vignaunguiculata. Nigerian Journal of Biochemistry and Molecular Biology, 17(1):51-56.

[5] Ezeonu, M., Okafor, J. andOgbonna, J. (2013).Laboratory Exercises in Microbiology.1st Edn. Ephrata Publishing and Printing Company, Nsukka. Pp 100-117.

[6] Gheytanchi, E., Heshmati, F., Kordestani, B., Nowroozi, J. and Movahedzadeh, F. (2010). Study on β-galactosidase enzyme produced by isolated Lactobacilli from milk and cheese. African Journal of Microbiology Research, 4(6), 454-458.

[7] Haider, T. and Husain, Q. (2007). Preparation of lactose free milk by using ammonium sulphate fractionated proteins from almonds. Journal of Food Science and Agriculture, 87: 1278–1283.

[8] Jacobson, R., Zhang, X., Dubose, R. and Matthews, B. (2014). Three-dimensional structure of β-galactosidase from E. Coli. Nature, 369 (6483): 761–766.

[9] Kim, C., Chung, H., Lee, M., Choi, L. and Kim, M. (2009). Development of dried liposomes containing beta-galactosidase for the digestion of lactose in milk. International Journal of Pharm., 183: 185–193.

[10] Kumar, S., Stecher, G. and Tamura, K. (2016). Molecular evolutionary genetics analysis version 7.0 for bigger datasets.Molecular Biology and Evolution, 33 (7): 1870- 1874.

[11] Lee, D., Kang, S., Suh, S. andByun, J. (2013). Purification and characterization of a β-galactosidase from peach (Prunuspersica). Molecules andCells, 15: 68–74.

[12] Loeffler, S., Sinnott, L., Sykes, D. and Withers, G.(2009).Interaction of the Lac Z β-galactosidase of Escherichia coli with some β-D-galactopyranoside competitive inhibitors. Biochemistry Journal, 177:145–152.

[13] Lowry, O., Roseburg, N., Farr, A. and Randall, R. (1951).Protein Measurement with Folin- Phenol Reagents. Journal of Biological Chemistry,93:265-275.

[14] Matthews, B. (2015). The structure of E. coli beta-galactosidase. ComptesRendus Biologies, 328 (6): 549–56.

[15] Picard, C., Fioramonti, J., Francois, A., Robinson, T., Neant, F. and Matuchansky, C. (2005). Review article: Bifidobacteria as probiotic agents -- physiological effects and clinical benefits. Alimentary Pharmacology and Therapy,22: 495–512.

[16] Rech, R. and Ayub, M. (2007).Simplified feeding strategies for fed-batch cultivation of Kluyveromycesmarxianus in cheese whey.Proceedings of Biochemistry, 42: 873–877.

[17] Tello-Solís, S., Jiménez-Guzmán, J., Sarabia-Leos, C., Gómez-Ruíz, L., Cruz- Guerrero, A., Rodríguez-Serrano, G. andGarcía-Garibay, M. (2005).Determination of the secondary structure of Kluyveromyceslactis β-galactosidaseby circular dichroism (CD) and its structure-activity relationship as a function of the pH. Journal of Agriculture and Food Chemistry,53: 10200–10204.

[18] Vasiljevic T. and Jelen P.(2001).Production of β- galactosidase for lactose hydrolysis in milk and products using thermophilic lactic acid bacteria. Journal of Innovation, Food Science and Emerging Technology,2: 75-85.