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ORIGINAL ARTICLE

Purification and characterization of β-galactosidase from *Aspergillus niger* PQ570689 for lactose hydrolysis and prebiotic synthesis

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Abstract

β-Galactosidase converts lactose to galactose and glucose, and it is significant for lactose-intolerant people. A Nigerian local strain of *Aspergillus niger* (accession number PQ570689) previously isolated from fermented goat milk in Minna, Niger State, Nigeria, was used to produce β-galactosidase. Diammonium sulfate precipitation and gel filtration were used to purify the crude enzyme. The impacts of temperature (10, 20, 30, 40, and 50 °C), pH (4, 5, 6, 7, and 8), metal ions (Fe, Mn, Cu, and Mg), chemical activators/inhibitors (SDS and Tween-80), sugars (fructose, glucose, and sucrose), and substrate concentrations (o-nitrofenyl-beta-D-galaktopyranoside (ONPG)) on the purified enzyme activity were determined. The kinetic parameters including Vmax (maximum velocity) and Km (Michaelis–Menten constant) of the partially purified enzyme were also estimated. Afterwards, lactose hydrolysis and prebiotic production potential of the purified beta-galactosidase was evaluated using high-performance liquid chromatography (HPLC). The purified enzyme was characterized by the highest enzyme activity (338 U/mL), specific activity (18.82 U/mL per mg), and fold purification (35.78), as well as the least protein content (18 mg/mL). The purified β-galactosidase was most active at pH 7 and 50 °C. The enzyme was, however, inhibited by all the metal ions, surfactants, and sugars tested. This biocatalyst was characterized by a substantial catalytic capability (Vmax = 666.67 U/mL per min) and moderate affinity (Km = 8.67 mmol) to its substrate. The purified enzyme showed a great potential for lactose hydrolysis (> 99%) as well as a good prospect for prebiotics synthesis (7%). Thus, the β-galactosidase can be developed for various industrial, environmental, and biotechnological applications.

Keywords β-Aalactosidase · *Aspergillus niger* · Lactose hydrolysis · Prebiotics synthesis

Introduction

Enzymes play a critical role in industrial biotechnology, offering eco-friendly and efficient solutions for various applications, including food processing, pharmaceuticals, and environmental management (Kuthiala et al. 2022). Among these biocatalysts, beta-galactosidase (EC 3.2.1.23, β-galactosidase) possesses a particular significance due to its ability to hydrolyze lactose into glucose and galactose, making it essential in the dairy industry (Movahedpour et al. 2022). As a glycoside hydrolase, the enzyme catalyzes the hydrolysis of β−1,4-galactosidic linkages present in lactose and other galactosides (Kaur et al. 2023). Additionally, it catalyzes trans-galactosylation reactions, leading to the synthesis of prebiotics galacto-oligosaccharides (GOS), which have been recognized for their health benefits, particularly in improving gut microbiota (Niharika and Ravindra 2025). Transgalactosylation is a reaction where a covalent glycosyl-enzyme intermediate is created and hydrolyzed by oxocarbenium ion-like transition states in a twofold displacement process (Kaur et al. 2023).

The production of beta-galactosidase from microbial sources has gained increasing attention due to its ease of cultivation, cost-effectiveness, and ability to generate highly stable and efficient enzymes under varying industrial

Highlights• *Aspergillus niger* was used to produce β-galactosidase enzyme.
- The enzyme had an activity of 338 U/mL and a specific activity of 18.82 U/mL per mg.
- The enzyme had a fold purification and protein content of 35.78 and 18 mg/mL.
- The enzyme was most active at pH 7 and 50 °C.
- The enzyme was highly efficient for lactose hydrolysis (> 90%).
- The enzyme showed a good prospect for prebiotics synthesis (7% yield).

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conditions (Kalathinathan et al. 2023; Kaur et al. 2023). Beta-galactosidase has wide-ranging applications in several industries (de Albuquerque et al. 2021), including dairy and other food industries where it is utilized to produce dairy products devoid of lactose suitable for lactose-intolerant individuals as well as the enhancement of sweetness and solubility of dairy products while reducing crystallization in condensed milk and ice cream (de Campos et al. 2023; Kaur et al. 2023; Niharika and Ravindra 2025). It is also employed in the synthesis of prebiotics, such as galacto-oligosaccharides (GOS), which are beneficial to the gut microbiota (Wang et al. 2024; Ignatova et al. 2025). In biotechnology and medicine, beta-galactosidase plays a significant role as a reporter enzyme and in therapeutic enzyme replacement therapies, respectively (Movahedpour et al. 2022; Ortega et al. 2025). In environmental biotechnology, beta-galactosidase is utilized in the treatment of dairy wastewater by breaking down lactose-rich effluents (Leong et al. 2021; Kaur et al. 2023). Microorganisms are a preferred source of β-galactosidase due to their rapid growth rates, genetic adaptability, and high enzyme yields (de Albuquerque et al. 2021; Movahedpour et al. 2022). Microorganisms secrete extracellular β-galactosidase into the surrounding medium to hydrolyze lactose externally, unlike intracellular β-galactosidase, which functions within the cell and requires substrate transport across the membrane (Kaur et al. 2023). *Aspergillus niger* has been extensively studied for its potential in producing several industrial products including extracellular beta-galactosidase (Idris et al. 2024; Ramasamy 2024; Badoni et al. 2025). Purified products obtained from A. niger are of great economic significance and are generally recognized as safe (GRAS) for human consumption, which is critical for food-related applications (Martarello et al. 2019). Fungal β-galactosidases typically exhibit high thermostability, acid tolerance, extracellular nature, and broad substrate specificity, making them superior to bacterial or yeast-derived enzymes in many applications (Panesar et al. 2016; Kaur et al. 2023).

Seventy percent of people worldwide experience health issues due to the consumption of lactose-rich foods (Martarello et al. 2019). As a result, lactose intolerance is a frequent health issue that causes gastrointestinal symptoms that include stomach cramping, pain, bloating, gurgling, or rumbling sounds in the stomach, in addition to frequent passing of gas, diarrhea or loose stools, and vomiting (Mohammed et al. 2022). As a result, individuals that have this condition avoid dairy products (Mohammed et al. 2022). Hence, beta-galactosidase is essential for the lactose-intolerant group because it produces lactose-free milk and other dairy products needed by many adults, without which, they cannot effectively digest dairy products (Ahmed et al. 2016).

The hygroscopic, poorly soluble property of lactose causes ease of crystallization; hence, it gives rise to a product with a sandy, mealy, or gritty texture, causing technological problems in certain dairy products (Ganta et al. 2022). The β-galactosidase hydrolytic ability of milk lactose therefore increases the digestibility, solubility, taste, and may improve functional properties of the dairy products (Kastsianevich 2017). Consequently, low-lactose foods are produced, which is perfect for people who are lactose intolerant (Volford et al. 2021). Beta-galactosidase can hydrolyze whey and other related dairy wastes as well as other lactose-rich foods to create simultaneously the monosaccharides, galactose, and glucose, as well as galacto-oligosaccharide (GOS) (Rocha and Guerra 2020). Keeping the above in view, this study therefore was carried out to produce, purify, and characterize extracellular beta-galactosidase from locally isolated *A. niger* (PQ570689) for industrial applications.

Materials and methods

Study area

This study was conducted in the Department of Microbiology, Federal University of Technology Minna, Niger State, Nigeria. Minna is the capital city of Niger State, located in the North Central part of Nigeria.

Source of fungus

Aspergillus niger (PQ570689) previously tested and confirmed to possess beta-galactosidase production potential was sourced from the Department of Microbiology, Federal University of Technology Minna, Niger State, Nigeria. The authors isolated the fungus from fermented goat milk collected from Tudun Fulani, Minna, Niger State Nigeria. The fungal isolate was screened qualitatively and quantitatively for beta-galactosidase production potential using Yeast Extract Peptone Lactose Broth (YEPLB) and o-nitrofenyl-beta-D-galaktopyranoside method, respectively (Idris et al. 2024).

Preparation of inoculum

The fungal isolate was sub-cultured on Potato Dextrose Agar (PDA) (supplemented with 40 g/L lactose) slants and incubated at 35 °C for 5 days. The PDA slants containing the fungal isolates were aseptically filled with 5.0 mL of 2.5% sterile Tween 80 using a sterile syringe. An inoculum size of 1.0×10^7 spores per mL was obtained by counting the fungal spores in a Neubauer chamber after the spores were dislodged and 5 mL of the spore suspension was diluted in 95 mL of sterile distilled water (Adhikari and Shrestha 1989). This was used as inoculum for the fermentation experiment (Antoine et al. 2015).

Production of beta-galactosidase by *Aspergillus niger* (PQ570689).

The enzyme production was done following the method of Al-Jazairi et al. (2015) with some modifications, including the organism used, the composition of the medium, the inoculum size, and the incubation conditions. One liter of Czapek-Dox broth containing (g/L) lactose (60), glucose (10), $NaNO_3$ (2.0), K_2HPO_4 (1.0), $MgSO_4.7H_2O$ (0.5), KCl (0.5), and $FeSO_4.5H_2O$ (0.01) was prepared in 2-L Erlenmeyer flasks. The pH was thereafter adjusted to 7. The flask containing the media was sterilized at 121 °C for 15 min in an autoclave.

The Erlenmeyer flasks holding 1 L of the sterile media were aseptically inoculated with 2.5 mL (1%) of the inoculum using a sterile syringe. The broth was incubated at 30 °C ± 2 °C for 7 days on a rotary shaker at 200 rpm for 8 h daily. Following the period of incubation, the broth was harvested and filtered via Whatman Number 1 filters to separate the fungal biomass from the filtrate. At 4 °C, the filtrate was centrifuged for 10 min at 10,000 rpm. Using a sterile Pasteur pipette, the supernatant was extracted from the pellet and transferred into sterile test tubes. After that, the solution was examined for beta-galactosidase activity and specific activity (Murugan 2013).

Determination of enzyme activity

Using a micropipette, exactly 750 μL of 2.0 mmol/L o-nitrofenyl-beta-D-galaktopyranoside (ONPG) (Sigma-Aldrich) substrate solution (pH 6.8) was transferred into a series of test tubes, and 250 μL of the crude beta-galactosidase was subsequently transferred into the tubes. Thereafter, the test tubes were filled with 2 mL of phosphate buffer (pH 6.8, 0.1 mol/L), and they were incubated for 40 min at 37 °C. Exactly 250 μL of 0.5 mol/L Na_2CO_3 was added to the mixture to stop the reaction. Reaction progress was determined by estimating the o-nitrophenol (ONP) liberated at a wavelength of 420 nm against the blank composed of ONPG (750 μL), phosphate buffer (2250 μL), pH 6.8, with the use of a spectrophotometer. After that, the enzyme's activity was determined using the formula.

β-galactosidase activity (U/mL) = (Dahal et al. 2020).

where A is absorbance at 420 nm; Trv is total reaction volume; E_v is enzyme volume; T is incubation period.

One unit of beta-galactosidase activity is the quantity of the enzyme needed to release 1 μmoL of ONP per min under assay conditions (Mowery and Seidman 2005; Murugan 2013; Antoine et al. 2015; Panesar et al. 2016). The fungal strains with the highest beta-galactosidase activities were selected for further studies.

Determination of total protein content

Protein concentration of the sample was determined using the Bradford technique with bovine serum albumin as standard protein (Mowery and Seidman 2005). The specific activity was thus determined using the equation: SA = Enzyme activity (U/mL)/Total proteins (mg/mL).

Purification of the crude β-galactosidase

Purification of the crude enzyme was done using the steps described by Mowery and Seidman (2005) and Princely et al. (2013). Unless otherwise noted, every purification process was done at ambient temperature (28 °C ± 2°).

Diammonium sulphate precipitation

A 50% volume increase was prepared by transferring the crude cell-free extract with beta-galactosidase activity into a 500-mL beaker equipped with a stirring bar. Throughout 30 to 45 min, crystalline diammonium sulfate was gradually added while being gently stirred in an ice bath. To reach a final concentration of 70% (w/v), 231 mg of diammonium sulfate per mL of starting solution was added and continuously stirred for 1 to 3 h, and 1 mL of 1 mol/L NaOH was added for every 10 g of diammonium sulfate. The sample was centrifuged at 10,000 rpm for 10 min, and the supernatant was separated from the precipitate. The precipitates were subsequently re-suspended in 1/10 the initial amount of 0.1 mol/L phosphate buffer.

Gel filtration

A 40 cm by 1.5 cm column was filled with Sephadex G-100 and equilibrated with phosphate buffer (0.1 mol/L, pH 7). The column was filled with the precipitated enzyme solution. The same buffer was used to elute the sample at a flow rate of 3 mL per h, and the fractions were collected to measure the protein content and enzyme activity. The fractions with beta-galactosidase activity were combined. Beta-galactosidase activity, specific activity, total protein, enzyme yield percentage, and purification fold were measured for every purification step. (Mowery and Seidman 2005).

Characterization of the partially purified β-galactosidase

The effects of pH, temperature, metal ions, chemical activators, and substrate concentrations on the purified enzyme activity were determined. These parameters were determined following the methods of Princely et al. (2013) and Cardoso et al. (2017). A microtiter plate and reader were used in the assay. Kinetic parameters, including Michaelis–Menten constant (Km) and maximum velocity (Vmax) of the purified enzyme, were also estimated.

Effect of pH on beta-galactosidase activity

The beta-galactosidase activity was investigated by incubating the enzymes in pH 4, 5, 6, 7, and 8 for 45 min to determine the optimum pH. Two different buffers were used; citrate buffer (0.05 mol/L) was used for pH ranges from 4.0 to 6.0, and sodium phosphate buffer (0.05 mol/L) was used for pH ranges from 6.0 to 8.0. The enzyme activities were determined thereafter using the standard assay procedure described earlier.

Effect of temperature on β-galactosidase activity

The extracellular β-galactosidase activity was assessed following incubation at various temperatures for 45 min to establish the optimum temperature (10, 20, 30, 40, and 50 °C). The enzyme activity was thereafter determined using the standard assay procedures (Basha et al. 2009).

Effect of metal ions on beta-galactosidase activity

The impact of metal ions on beta-galactosidase activity was assessed by mixing several metal salts with 50 mmol/L sodium citrate buffer (pH 4.5). In every instance, solutions having a final concentration of 10 mmol/L were produced. Thereafter, 50 µL of the pure enzyme solution and 50 µL of ONPG solutions (3 mmol/L) were added to 100 µL of the different salt solutions. For 10 min, the mixtures were incubated at 35 °C. Following the incubation time, the enzyme activities were measured in each instance. The metal salts chosen for this investigation were $MgCl_2$, $FeSO_4$, $MnSO_4$, and $CuSO_4$.

Effect of chemical activators and inhibitors on β-galactosidase activity

To investigate their effects as activators or inhibitors on the purified enzyme, sugars, detergents, and additives were introduced to sodium-citrate buffer (50 mmol/L, pH 4.5). In each instance, solutions with final concentrations of 10 mmol/L were produced. Different buffer solutions containing Tween-80 (1% w/v) and sodium dodecyl sulfate (SDS, 10 mmol/L) were made for the detergents. Different fructose, glucose, and sucrose concentrations (5, 25, 75, and 100 mmol/L) in sodium-citrate buffer were utilized as sugars. Thereafter, 50 µL of the pure enzyme solution and 50 µL of ONPG solution (3 mmol/L) were added. For 10 min, the mixtures were incubated at 35 °C. After the incubation period, the enzyme activity was measured in each instance.

Effect of substrate concentration on β-galactosidase activity

Various ONPG concentrations (1, 5, 10, 15, 20, 25, and 30 mmol/L) in 0.1 mol/L phosphate buffer at pH 7 were prepared and used to observe the effect of substrate concentration on β-galactosidase activity using the purified enzyme solution. The mixtures were incubated at 35 °C for 10 min, and the beta-galactosidase activity was measured following the incubation period.

Determination of Km (Michaelis–Menten constant) and Vmax (maximum velocity)

The maximum velocity (Vmax) and Michaelis–Menten constant (Km) values were determined using a Lineweaver–Burk/double reciprocal plot. Plotting 1/Vo against 1/[S] produced a straight line with an intercept on the Y-axis, or 1/Vmax, with a slope of Km/Vmax and an intercept on the *X*-axis's negative side. The graph was used to compute the Vmax and Km.

Lactose hydrolysis and prebiotic synthesis potential of the beta-galactosidase

The potential of the purified β-galactosidase to hydrolyze lactose and produce prebiotics was examined. In sterile test tubes, 5 mL of the pure enzyme and 5 mL of the sugar solution (300 g/L lactose + 300 g/L fructose for lactulose formation) made in sodium-citrate buffer (50 mmol/L, pH 4.5) were combined to perform the enzymatic synthesis for 5 h at 37 °C. After the incubation period of 1, 2, 3, 4, and 5 h, the sample was collected. The samples were then deproteinized by adding 1 mL of 10% trichloroacetic acid (TCA) to 5 mL of the samples. The samples were centrifuged for 10 min at 5000 rpm; the supernatant was extracted from the pellet and transferred into sterile sample bottles using a sterile pipette. Following that, HPLC was used to determine the lactose and lactulose concentrations in the samples (supernatant).

HPLC analysis for determination of lactose and lactulose concentration

The HPLC analysis for the determination of lactose and lactulose concentrations of the samples was carried out in the Chemistry Advanced Research Centre (CARC), Sheda Science and Technology Complex (SHESTCO), Abuja. Different concentrations (1 to 400 g) of lactose and lactulose standards were prepared and used to calibrate the HPLC machine (Cecil 4100 with UV detector). Thereafter, 20 µL of the sample was injected into the column (c-18 ODS 450 × 4.6 mm) using a Rheodyne Model 7105 syringe loading sample injector, and eluted with methanol at a flow rate of 1 mL/min for determination of lactose concentration. A wavelength of 283 nm for 5 min was used for estimation of lactose concentration. For determination of lactulose concentration, 20 of the sample was injected into the column (c-18 ODS 450×4.6 mm) and eluted with acetonitrile/water (25:75 v/v) at a flow rate of 1 mL/min. The wavelength used for the determination of lactulose concentration was 385 nm for 15 min (Rentschler et al. 2016). The lactose conversion and the prebiotic yield were thus calculated using the equations below.

$$\text{Lactose Conversion} = \frac{Ci - Cf}{Ci} \times 100$$

$$\text{Prebiotic yield} = \frac{Cp}{Ci} \times 100$$

where Ci and Cf are the initial and final concentrations of lactose, respectively, and Cp is the concentration of prebiotics (lactulose) (Cardoso et al. 2017).

Data analysis

Data obtained was expressed as means ± standard deviation and presented in tables, graphs, plates, or charts where necessary. Significantly different values were separated using the Microsoft Excel Version 2007 package.

Results

The activity, total protein, specific activity, purification fold, and percentage yield of each purification step (fraction) of the *Aspergillus niger* (PQ570689) beta-galactosidase is presented in Table 1. An increase in enzyme activity from 29.20 to 110.40 and 338.70 U/mL for each purification step was observed. A two-fold increase from 96.2 to 209.9 mg/mL in total protein was observed on precipitation with diammonium sulfate, followed by a remarkable decrease after gel filtration to 18 mg/mL. Specific activities of the enzyme increased from 0.30 to 0.53 and 18.82 U/mL per mg for each purification step. An increase in the fold purification of the enzyme was also observed from 1 to 35.78, while

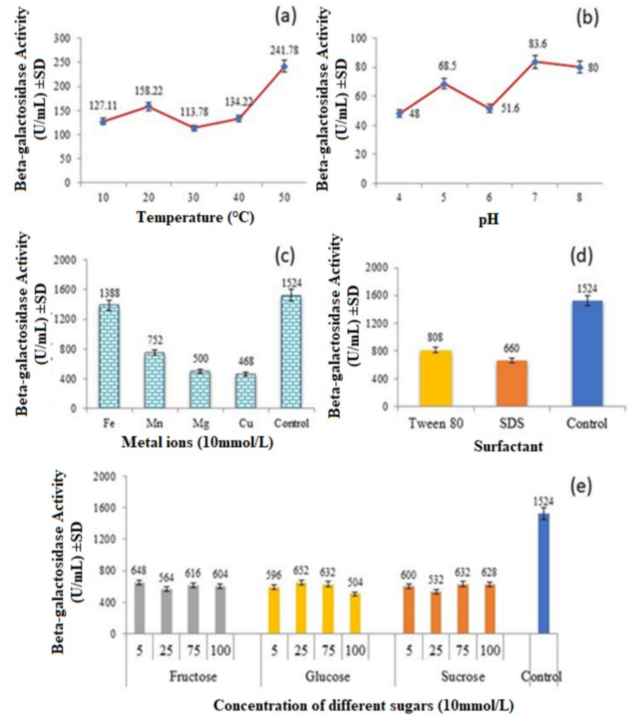

Fig. 1 Effect of temperature (**a**), pH (**b**), metals (**c**), surfactants (**d**), and different concentrations of sugars (**e**) on the β-galactosidase activity

the percentage yield also increased from 100 to 378.08% on purification with diammonium sulfate. A slight decrease was thereafter observed (306.79%) when purified using gel filtration.

The effect of temperature, pH, metals, surfactants, and different concentrations of sugars on the beta-galactosidase activity is presented in Fig. 1. Effect of temperature on *Aspergillus niger* (PQ570689) beta-galactosidase activity reveals an initial increase in the activity from 127.11 to 158.22 U/mL for temperatures 10 and 20 °C then followed by a dramatic decrease in enzyme activity to 113 U/mL for 30°C. The activities thereafter increased to 134.22 and 241.78 U/mL for 40 and 50 °C, respectively. The highest beta-galactosidase activity of 83.6 U/mL was observed at pH 7, followed by pH 8, pH 5, and pH 6 with enzyme activities of 80, 68.5, and 51.6 U/mL, respectively. The least beta-galactosidase activity of 48 U/mL was seen at pH 4. When

Table 1 Purification table for *Aspergillus niger* (PQ570689) β-galactosidase

Enzyme fraction/step	Activity (U/mL)	Total protein (mg/mL)	Specific activity (U/mL per mg)	Purification fold	% Yield
Crude	29.20	96.20	0.30	1.00	100.00
Diammonium sulfate precipitation	110.40	209.9	0.53	1.73	378.08
Gel filtration	338.70	18.00	18.82	35.78	306.79

compared to the control (1524 U/mL), all the metal ions studied reduced the enzyme activity, with Fe (1388 U/mL) having the least effect on the enzyme activity, followed by Mn, Mg, and Cu with mean enzyme activities of 752, 500, and 468 U/mL, respectively.

Similarly, both surfactants (Tween 80 and SDS) investigated also reduced the beta-galactosidase activity, with Tween 80 (808 U/mL) having the least effect on the enzyme activity, while SDS (660 U/mL) greatly reduced the activity of the enzyme when compared to the control (1524 U/mL). The effect of different concentrations of different sugars on the enzyme activity shows that all the sugars studied reduced the enzyme activity at different concentrations (5 to 100 mmol/L) when compared to the control with enzyme activity of 1524 U/mL. The activity of the enzyme in the presence of fructose ranged from 564 to 648 U/mL, while in the presence of glucose, the enzyme activity ranged from 504 to 652 U/mL. A range of 532 to 632 U/mL beta-galactosidase activities was observed for the enzyme in the presence of sucrose.

For the effects of substrate concentration on the activity of the enzyme, as seen in Table 2, an increasing beta-galactosidase activity from 69.40 to 808 U/mL was observed with increasing concentration of the substrate (ONPG) from 1 to 20 mmol/L. The enzyme activity however decreased from 808 to 648 U/mL with further increase in the substrate concentration from 20 to 30 mmol/L. Lineweaver–Burk/double reciprocal graph (Fig. 2) plotted using data (1/[S] and 1/V) from Table 2 shows the straight-line graph with R^2 (0.9684), linear equation ($y = 0.0132x + 0.0015$). The Km and Vmax extrapolated from the graph were 8.67 mmol/L of ONPG and 666.67 U/mL per min, respectively, shown in Table 3 along with other kinetic parameters of the enzyme including total protein (18 mg/mL), optimum pH (7), optimum temperature (50° C), enzyme activity (338.70 U/mL), and specific activity (18.82 U/mL per mg).

The purified beta-galactosidase showed a significantly high lactose hydrolysis for all the five incubation periods examined. There was no significant difference

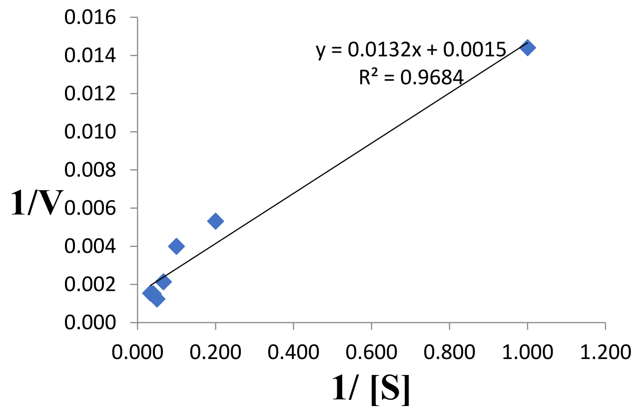

Fig. 2 Lineweaver–Burk double reciprocal plot

(p-value > 0.05) between the percentage of lactose hydrolysis for all the incubation periods. A 99.8% lactose hydrolysis, which had a final lactose concentration of 0.6897 mg/mL, was observed for a 4-h incubation period, which was closely followed by 99.7% lactose hydrolysis for both 2- and 3-h incubation periods, with final lactose concentrations of 0.7686 and 1.0334 mg/mL, respectively. Meanwhile, 99.6% was observed for 1- and 5-h incubation periods with final concentrations of lactose 1.3488 and 1.2575 mg/mL, respectively (Table 4; Supplementary Data (SD) 1–5).

The highest prebiotics synthesized by the enzyme was observed at 4 h incubation, which produced a lactulose concentration of 1.0571 mg/mL translating to 7.05%. This was followed by 5- and 3-h incubation periods, which elicited lactulose concentrations of 0.6168 (4.11%) and 0.5460 mg/mL (3.64%), respectively. The least prebiotics synthesized by the enzyme was observed at incubation periods 2 and 1 h, which produced lactulose concentrations of 0.4451 and 0.0710 mg/mL translating to 2.97 and 0.47%, respectively. The prebiotic synthesis by the purified enzyme is presented in Table 5 and SD 6–10.

Table 2 Effect of substrate concentration on the beta-galactosidase activity

Substrate concentration (mmol/L)	β-galactosidase Activity (U/mL)	1/[S]	1/V
1	69.4	1.000	0.014
5	188	0.200	0.005
10	250	0.100	0.004
15	468	0.067	0.002
20	808	0.050	0.001
25	660	0.040	0.002
30	648	0.033	0.002

Table 3 Kinetic parameters of partially purified beta-galactosidase

Enzyme parameter	Value
Total protein of enzyme	18 mg/mL
Optimum pH	7
Optimum temperature	50 °C
Km	8.67 mmol/L of ONPG
Vmax	666.67 U/mL per min
Enzyme activity	338.70 U/mL
Specific enzyme activity	18.82 U/mL per mg

Table 4 Chromatographic parameters for lactose hydrolysis by the purified beta-galactosidase

Peak no	Peak name (incubation)	Ret. time (min:s)	Area (m^2)	Height (m)	Lactose conc. (mg/mL)	Lactose hydrolysis (%)
037	1 h	01:26.6	35.4	10.8	1.3488	99.6
050	2 h	01:27.6	20.2	6.9	0.7686	99.7
038	3 h	01:28.1	27.1	8.4	1.0334	99.7
051	4 h	01:26.9	18.1	6.9	0.6897	99.8
035	5 h	01:27.1	33.0	9.2	1.2575	99.6

Table 5 Chromatographic parameters for prebiotics synthesized by the purified Beta-galactosidase

Peak no	Peak name (incubation)	Ret. time (min:s)	Area (m^2)	Height (m)	Lactulose conc. (mg/mL)	Prebiotic synthesis (%)
018	1 h	05:04.2	0.1	0.1	0.0710	0.47
016	2 h	04:58.3	0.4	0.3	0.4451	2.97
022	3 h	05:01.6	0.0	0.1	0.5460	3.64
011	4 h	04:51.7	1.0	0.6	1.0571	7.05
017	5 h	04:48.2	0.6	0.2	0.6168	4.11

Discussion

According to Martarello et al. (2019), over 70% of individuals globally suffer from health problems as a result of eating food products high in lactose. Therefore, lactose intolerance is a common health problem that results in frequent gas, diarrhea or loose stools, vomiting, and other gastrointestinal problems. Consequently, people with this condition stay away from dairy products (Mohammed et al. 2022). β-galactosidase is therefore crucial for the lactose-intolerant group because it creates lactose-free milk and other dairy products that many lactose-intolerant adults worldwide require. Without this enzyme, they cannot efficiently digest dairy products (Ahmed et al. 2016).

In this study, β-galactosidase was purified and characterized from *Aspergillus niger* PQ570689, revealing its potential for effective lactose hydrolysis and prebiotics synthesis. The reduction in total proteins and increase in β-galactosidase activity, specific activity, fold purification, and enzyme yield during purification using diammonium sulfate precipitation and gel filtration could be due to the selective concentration and stabilization of the enzyme, as well as the effective removal of impurities and preservation of enzyme integrity and activity under non-denaturing conditions. These purification steps may have enhanced the purity and performance of the enzyme (Volford et al. 2021). The enhanced β-galactosidase activity, specific activity, fold purification, and enzyme yield achieved through diammonium sulfate precipitation and gel filtration may lead to improved efficiency, cost-effectiveness, enzyme quality, application versatility, stability, regulatory compliance, and reduced environmental impact. These benefits could make the enzyme more attractive and practical for various industrial applications, particularly in the food, pharmaceutical, and biotechnology sectors. The finding of the research agrees with the results of Kamran et al. (2017). Martarello et al. (2019); Bano et al. (2021); Vidya et al. (2020a), and Vidya et al. (2020b) in their separate studies reported a reduction in total proteins, increased specific activity, and fold purification of the β-galactosidase isolated from *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus awamori*, and *Aspergillus terreus*, respectively. Meanwhile, the separate studies of Martarello et al. (2019) and Vidya et al. (2020b) reported decreasing β-galactosidaseactivities and percentage yield with increasing purification step for enzyme produced by *Aspergillus niger* and *Aspergillus terreus*, which disagrees with the findings of this research. This disagreement could be attributed to the source or nature of the enzyme or the purification steps/methods used.

The data from this study revealed a non-linear relationship between temperature and enzyme activity. *Aspergillus niger* (PQ570689) beta-galactosidase was most active at higher temperatures, with 50 °C being especially beneficial. This ideal temperature most likely reflects the native conditions of the enzyme, or where it has evolved to perform at its best. The enzyme started to lose catalytic efficiency beyond a certain temperature range, as observed in the activity decline at 30 °C. The reduction in β-galactosidase activity at 30 °C may be due to decreased molecular kinetics, suboptimal activation energy, reduced active site flexibility, source-specific temperature preferences, or partial enzyme inactivation (Kaur et al. 2023). For industrial applications, especially in lactose hydrolysis and prebiotics synthesis operations for dairy products, it is essential to comprehend how temperature affects β-galactosidase activity. Maintaining a temperature close to the ideal 50 °C can greatly improve both the yield and process efficiency. The result of this study agrees with the findings of Martarello et al. (2019) and Bano et al. (2021) who in their separate investigations reported β-galactosidase isolated from *Aspergillus niger* and *Aspergillus fumigatus*, respectively, having maximum activities at 50 °C. This study disagrees with the findings of Vidya et al. (2020a), who reported maximum enzyme activity at 25 °C. These disagreements could be attributed to the source and nature of the enzyme or the methodology used.

The result of this investigation also revealed a non-linear correlation between pH and enzyme activity. The data suggest that beta-galactosidase activity is quite sensitive to pH variations, with a distinct optimal pH for maximum activity. According to the pattern of enzyme activity observed in this study, the ideal pH for *Aspergillus niger* β-galactosidase is approximately 7. This may be attributed to the enzyme's active site requiring particular ionization states, which could have been affected negatively by both very acidic and slightly acidic environments, based on the dramatic reduction in activity at pH 4 and pH 6. The enzyme may operate in an alkaline environment, although its ideal structure and charge distribution are reached at a neutral pH, as seen by the modest drop in activity at pH 8. Consequently, for industrial processes involving β-galactosidase, such as lactose hydrolysis in dairy products, maintaining neutral pH may be crucial for maximizing enzyme efficiency as well as product yield. Operating at the enzyme's optimal pH ensures the highest catalytic performance by reducing the amount of enzyme needed, which may reduce production cost. This result agrees with the studies of Bano et al. (2021) who also reported maximum β-galactosidase activity at neutral pH (7). Meanwhile, the studies of Martarello et al. (2019) reported highest *Aspergillus niger* β-galactosidase activity at an acidic pH of 3. This variation could be attributed to the enzyme source (i.e., fungal strain), or the methodology used.

All the metal ions tested for their effects on the enzyme showed a negative influence on the activity. This is evidenced in the reduction of enzyme activity as compared to the control. The results suggest that β-galactosidase from *Aspergillus niger* (PQ570689) is highly sensitive to the presence of metal ions, with varying degrees of inhibition depending on the ion type. The control exhibited the highest activity, indicating that the native enzyme structure and function are optimal without the addition of these metal ions. Iron showed the least inhibition, possibly due to weaker interactions or less significant conformational changes induced by the ion. In contrast, manganese, magnesium, and especially copper significantly inhibited enzyme activity, likely through stronger binding and more disruptive interactions with the enzyme's active site or overall structure. Understanding the effects of these metal ions on beta-galactosidase activity is crucial for industrial processes where metal ions may be present. The inhibitory effects of manganese, magnesium, and copper highlight the need for careful control of metal ion concentrations in the production environment to maintain optimal enzyme activity. This would be particularly important in processes like lactose hydrolysis in dairy products, where enzyme efficiency directly impacts productivity and cost-effectiveness. The finding of this study agrees with investigations of Vidya et al. (2020b), who also reported the inhibition of *Aspergillus terreus* β-galactosidase by these ions. Meanwhile, the study of Bano et al. (2021) reported no effect of these ions on *Aspergillus fumigatus* beta-galactosidase, thereby disagreeing with the findings of this research. This disparity could be attributed to the source and nature of the enzyme or the difference in methodology used.

The presence of Tween 80 and SDS significantly inhibits the activity of β-galactosidase from *Aspergillus niger*, with SDS causing the most substantial reduction. The enzyme operates optimally in the absence of these substances, as indicated by the highest activity in the control. These findings highlight the importance of managing the presence and concentration of surfactants and detergents in industrial processes to maintain maximum enzyme efficiency and stability. The effect of these surfactants on β-galactosidase activity is crucial for its industrial application, particularly in processes where these substances might be present or used intentionally. The substantial reduction in activity with SDS highlights the need for careful selection and control of additives in processes that require the use of the enzyme. In industries like food processing, pharmaceuticals, and biotechnology, where enzymes might be exposed to detergents, it is crucial to choose conditions that minimize enzyme inactivation. In situations where surfactants, such as Tween 80, are utilized to improve solubility or stabilize emulsions, it is critical to balance the advantages of surfactants with the possibility of their inhibiting effects on enzyme activity by optimizing concentrations. This result agrees with the findings of Kamran et al. (2017) who also reported inhibitory effects of selected detergents, including SDS and Tween 80, on beta-galactosidase produced by *Aspergillus nidulans.* The findings of this research disagree with the investigation of Bano et al. (2021), who reported stabilizing and stimulatory effects of SDS and Tween 80 on β-galactosidase. This disagreement could also be attributed to the source and nature of the enzyme or the methodology used.

All tested sugars at all concentrations caused a significant reduction in enzyme activity compared to the control (1524 U/mL), indicating a general inhibitory effect of these sugars on beta-galactosidase activity. This could be due to competitive inhibition, allosteric effects, and/or substrate interference by these sugars on the enzyme. It is essential, therefore, to comprehend how these common sugars limit beta-galactosidase activity to apply the knowledge in industries like food processing, especially when developing lactose-free products where these sugars may be present. β-galactosidase activity increased with an increase in substrate concentration up to a point (20 mmol/L) where it reaches its peak. Beyond this concentration, the activity decreases, likely due to enzyme saturation or substrate inhibition. The Lineweaver–Burk plot of the beta-galactosidase activity data shows a strong linear relationship, as indicated by the regression equation $y = 0.0132x + 0.0015$ and a high R^2 value of 0.9684. This suggests that the enzyme follows typical Michaelis–Menten kinetics under the experimental conditions. The maximum velocity (666.67 U/mL) obtained in this study indicates the highest rate at which beta-galactosidase can convert the substrate to product when the enzyme is fully saturated with the substrate. Meanwhile, the Km (8.8 mmol/L) indicates a moderate affinity of β-galactosidase from *Aspergillus niger* for its substrate. These parameters could be valuable for optimizing the use of the enzyme in industrial applications, which may ensure efficient substrate utilization and high catalytic activity. The strong linear relationship and high R^2 value indicate reliable data and support the conclusions drawn about the enzyme's kinetic behavior.

The characterized beta-galactosidase produced by *Aspergillus niger* (PQ570689) shows optimal activity at pH 7 and 50 °C, with a Km of 8.67 mmol/L for ONPG and a high Vmax of 666.67 U/mL per min. The enzyme exhibits a substantial specific activity of 18.82 U/mL per mg, highlighting its efficiency. These parameters suggest that beta-galactosidase is well-suited for industrial applications requiring efficient catalysis under neutral pH and moderately high temperature conditions.

The near-complete hydrolysis (around 99.6 to 99.8%) indicates that the enzymatic activity is highly efficient. Such high levels of hydrolysis suggest that the enzyme is very effective under the given conditions. The slight fluctuations in the final lactose concentration and the percentage hydrolysis might indicate minor variations in enzyme activity or stability over time. However, these variations are minimal, suggesting that the enzyme retains its activity well over the 5 h. The data imply that such an enzymatic process could be highly effective for industrial applications, such as the production of lactose-free milk and dairy products. The consistent and high efficiency of lactose hydrolysis by the enzyme may be crucial for processes requiring precise and complete conversion of lactose, such as in fermentation processes or in the production of galacto-oligosaccharides (GOS). The findings of this study are in agreement with the research of Bella et al. (2022), who reported a maximum lactose hydrolysis of 86.21% by beta-galactosidase produced by *Aspergillus oryzae*. Meanwhile, the findings of Cardoso et al. (2017) and Nag et al. (2021) reported a maximum lactose conversion of 38% and 5% by β-galactosidase produced by *Aspergillus lacticoffeatus* and *Kluyveromyces* sp., respectively, which disagrees with the findings from this study. The disparity could be attributed to the difference in fungal isolate, substrate, or the method used.

The data on lactose hydrolysis indicate that the purified beta-galactosidase from *Aspergillus niger* (PQ570689) show variable prebiotics synthesis activity over time. The enzyme is capable of high synthesis rates, particularly around the 4 h mark, indicating its potential for effective prebiotic production in industrial applications. The peak at 4 h suggests that this is the optimal time for maximum prebiotics synthesis under the experimental conditions used. For industrial applications, maintaining these conditions could therefore be critical for maximizing yield. The fluctuations observed could be due to several factors, including changes in enzyme stability, substrate concentration, and product inhibition. Understanding these factors could help in optimizing the conditions for consistent and high-yield prebiotic synthesis.

The ability to synthesize prebiotics effectively is valuable for the food and pharmaceutical industries. Prebiotics are known for their health benefits, including promoting gut health by stimulating the growth of beneficial bacteria. The result of this study thus suggests that *Aspergillus niger* (PQ570689) β-galactosidase could be a promising candidate for such applications. The findings of this research agree with the reports of Cardoso et al. (2017) and Silvério et al. (2018), who revealed in their separate investigations 5% and 3.3% maximum prebiotic yield by *Aspergillus lacticoffeatus* and *A. restrictus* beta-galactosidases, respectively. This result disagrees with the findings of Volford et al. (2021), who reported a maximum prebiotic yield of 0.032% by β-galactosidase produced by *Rhizomucor pusillus*. This disparity could be attributed to the source of the enzyme.

Conclusion

The produced enzyme fraction purified after gel filtration was characterized by maximum enzyme activity, specific activity, and fold purification, as well as the lowest protein content. The purified enzyme was most active at neutral pH and moderately high temperature. The protein was, however, inhibited by all the metal ions, surfactants, and sugars tested. The purified beta-galactosidase was highly efficient for lactose hydrolysis with a near-total (> 90%) lactose conversion over the 5 h incubation period. The purified enzyme is a good prospect for prebiotics synthesis of up to 7% yield, observed after 4 h of incubation. This research suggests potential applications of the enzyme in formulations for lactose-intolerant individuals as well as for the production of prebiotics. Future research is needed to ensure that the efficient lactose hydrolysis (> 90%) and prebiotics synthesis (at the 7.05% level) of the purified beta-galactosidase can be consistently replicated on a larger scale. In other words, pilot-scale trials could help to translate this research into industrial-scale applications of the enzyme. The robust performance of the purified beta-galactosidase in lactose hydrolysis as well as prebiotics synthesis could be suitably employed for various industrial, environmental and biotechnological applications.

Abbreviations

***SDS*: Sodium dodecylsulphate; *ONPG*: O-nitrofenyl-beta-D-galactopyranoside; *HPLC*: High-performance liquid chromatography; *GRAS*: Generally recognized as safe; *YEPLB*: Yeast extract peptone lactose broth; *PDA*: Potato dextrose agar; *ONP*: O-nitrophenol; *SA*: Specific activity; *TCA*: Trichloroacetic acid; *CARC*: Chemistry Advanced Research Centre; *SHESTCO*: Sheda Science and Technology Complex**

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Data availability The nucleotide sequence of the isolate has been deposited in the NCBI database under the accession number PQ570689 and is available at the following link: https://www.ncbi.nlm.nih.gov/nuccore/PQ570689.1/.

Declarations

Conflict of interest The authors declare no competing interests.

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