Research article

Fourier transform infrared spectroscopy analysis of Lactose hydrolysis by beta-galactosidase from *Lactiplantibacillus plantarum* GV54 and *Lactiplantibacillus* sp. GV66

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ABSTRACT

Introduction and Aim: The enzyme beta-galactosidase is extremely important in the food and pharmaceutical industries. The goal of this work was to improve the lactose hydrolysis process employing beta-galactosidase to convert glucose and galactose for alleviating lactose intolerance.

Methods: Intracellular beta-galactosidase was extracted from *Lactiplantibacillus spp.* (GV54 and GV66) by cell permeabilized method using toluene/acetone solvents. Lactose hydrolysis was performed using 50 g/L of lactose with partially purified beta-galactosidase enzyme at different time intervals 2, 4, 6 and 12 h. Fourier transforms infrared spectroscopy (FTIR) was used to evaluate the degree of hydrolysis at different time intervals and lactose hydrolysis was high at 6 h of incubation.

Results: The FTIR peak of lactose molecules hydrolysis by beta-galactosidase (GV54 and GV66) with different time intervals (2h, 4h, 6h, and 12h) indicates similarities in bond lengths and vibrational modes. The amide I group of beta-galactosidase is uniformly bound to lactose at 1634 per cm and C-H group of glucose and galactose was observed at 1386 per cm. In the range of 2000-800 per cm, we could identify variations in the shape and strength of these peaks, compared with control (lactose solution).

Conclusion: The precise and direct measurement of individual carbohydrates such as glucose and galactose in lactose solution hydrolyzed by beta-galactosidase enzyme was successfully accomplished using FTIR spectrometry. Comparing FTIR analysis to traditional procedures, the time required is drastically decreased. These findings have the analytical power to follow the dynamics of *in vitro* enzymatic activity of naturally occurring substrates rich in natural carbohydrate polymers.

Keywords: FTIR; lactose; beta-galactosidase; *Lactiplantibacillus plantarum*; lactose intolerance.

INTRODUCTION

Lactose (β-D-galactopyranosyl-(1→4)-D-glucose) is a disaccharide carbohydrate made up of glucose and galactose monosaccharides, which form a β-(1,4) glycosidic linkage. Lactose is present in milk and other dairy products to provide texture, flavor, and adhesive characteristics and it is commonly used as an excipient in tablets in the pharmaceutical sector. Since the majority of adults worldwide cannot tolerate the lactose in ordinary milk products, usage of lactose-reduced milk and milk products also known as low lactose or lactose-free milk products are of particular economic interest (1, 2). Lactose intolerance is the inability to digest lactose due deficiency or absence of β-galactosidase (lactase) in the small intestine. β-galactosidase helps in hydrolysis of disaccharide lactose into the monosaccharides -glucose and galactose. Milk is treated with β-galactosidase sourced from lactic acid bacteria (LAB), to provide lactose-free milk (2, 3). β-galactosidase (EC 3.2.1.23) is the most essential enzyme in the dairy industry to produce low-lactose products to address lactose intolerance, which is produced commercially from microorganisms like bacteria, yeast, and fungi. It is preferable to employ bacterial enzymes due to their high activity and stability (2, 4). In order to avoid lactose crystallization and improve sweetness and milk product solubility, the enzyme β-galactosidase is used in industry as it hydrolyzes lactose into glucose and galactose. Temperature, enzyme concentration, and reaction inhibition are the main factors that affect the enzymatic reaction proceeds (3, 4). *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus,* and *Enterococcus* are a few genera of LAB. They are the primary fermentation starters, particularly for dairy products, and some of them are naturally present in

the gut microflora. They are considered as probiotics and organisms that are generally recognized as safe (GRAS) (5-7). The probiotic LAB is employed to treat intestinal issues, and that treatment has been helpful in animal models of numerous clinical intestinal disorders (8, 9).

Synthetic β-galactosidase is expensive, unsuitable for use in food, and insufficiently manufactured for industrial application. Therefore, there is a direct need to choose microorganisms safe for human consumption and capable of generating adequate amounts of β-galactosidase. For functional food applications, LAB are safe organisms, and have been observed to be the most desirable sources of β-galactosidase (10-12). The enzyme β-galactosidase is used in various industrial and medical applications. In addition to treating newborns with intestinal lactase deficiency caused by genetics, its medicinal characteristics can also are employed to avoid lactose crystallization in concentrated or frozen dairy products such as condensed milk and ice cream, increasing sensory quality and customer approval. Potential uses for β-galactosidase include the food industries, bioremediation, biosensor, and the detection and treatment of a variety of illnesses (13-15). The β-galactosidase enzyme perhaps used to create a biosensor to measure the amount of lactose in milk. It is also used to diagnose diseases and treat lactose intolerance. In addition, lactose hydrolyzed milk has been produced with the enzyme encapsulated (14, 15).

FTIR is one of the most used techniques for identifying chemical compounds and deciphering biochemical structures. Based on the IR profiles' fingerprinting properties, the food sector has used it to evaluate the quality of food. The typical chemical components of the milk powder (such as fat, protein, and carbohydrates) may be identified by analyzing the distinctive peaks of chemical functional groups (16). FTIR spectroscopy is described as fully automated for the rapid measurement of lactose in milk and other carbohydrates in soft drinks and also confirmed the degradation of the carbohydrate polymer (13-17). The goal of the current work was to study lactose hydrolysis by β-galactosidase from *Lactiplantibacillus* isolates by employing FTIR spectral analysis.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals were purchased from Himedia Laboratories Pvt, Ltd., India. Lactose was used as substrate for hydrolysis study, K_2HPO_4 and KH_2PO_4 were used for preparation of phosphate buffer (0.1 M) and used this buffer for the preparation of lactose and enzyme solutions; sodium carbonate ($NaCO_3$) was used to stop the reaction.

Production and extraction of β-galactosidase enzyme

A potential probiotic β-galactosidase producing Lactiplantibacillus spp. (GV54 and GV66) were characterized and identified by 16S rRNA gene sequencing in our previous study and were chosen for lactose hydrolysis study using FTIR. MRS (de Man Rogosa Sharpe) culture medium was supplemented with 4% lactose, used for the production of β-galactosidase. Selected isolates were inoculated with 100 mL of MRS broth with continuous shaking for 48h at 37°C. Further, centrifuged at 12,000 × g at 4ºC for 5 min and suspended in phosphate buffer (pH 6.8). The extraction of enzymes by cell permeabilized method using toluene/acetone solvents, 10 mL of cell suspension was treated with 0.1 mL of toluene/acetone (1:9 v/v), vortex for 7 min (18). Further cell free supernatant (CFS) was precipitated with ammonium sulfate using a magnetic stirrer until it reached a saturation level of 70% (w/v). After that precipitate was centrifuged at 12,000 × g, for 10 min at 4°C using 0.1 M phosphate buffer and the obtained precipitate was dialyzed overnight against a phosphate buffer (19, 20).

Lactose hydrolysis by β-galactosidase

Lactose hydrolysis was determined using 10 mL of lactose solution (50 g/L) as a substrate and 1 mL of partially purified β-galactosidase enzyme. Initially, β-galactosidase enzyme was added to lactose solution at 0 h and incubated at different time intervals 2, 4, 6, and 12 h. After 2 h incubation, 1 mL of aliquot was withdrawn and 0.5mL of 1M Na_2CO_3 was added to each tube to stop the reaction (21). Further, samples were collected at every 2 h for FTIR analysis to measure lactose hydrolysis (13).

FTIR analysis

Analysis of functional groups and chemical bonds in a molecule of different samples was determined by FTIR spectroscopy. FTIR spectra show variable peaks as a function of wave number (cm^{-1}). In this study, FTIR spectroscopy (Bruker Alpha II FTIR spectrophotometer with a ZnSe Crystal ATR) was performed to analyze lactose hydrolysis by β-galactosidase enzymes in lactose solution samples. Each spectrum was recorded between 4000 and 600 cm^{-1} to evaluate conversion of lactose (disaccharide) to galactose and glucose (monosaccharide) by β-galactosidase enzyme. Origin Software was used to process the spectra.

RESULTS

Lactose hydrolysis was accomplished using β-galactosidase at various time intervals. β-galactosidase hydrolyzes more than 50% of the lactose in 4 h, reaching a maximum at 6 h before reaching steady state. However, after 12 h, the rate of lactose hydrolysis by β-galactosidase began to decrease. Fig. 1 illustrated that the functional groups on lactose, β-galactosidase, glucose, and galactose were identified using FTIR spectroscopy. The vibration modes of enzyme's -NH and O-H groups created in between 3000 and 3500 cm^{-1}. The peaks seen at 3308 cm^{-1} indicate the -NH and O-H groups of β-galactosidase.

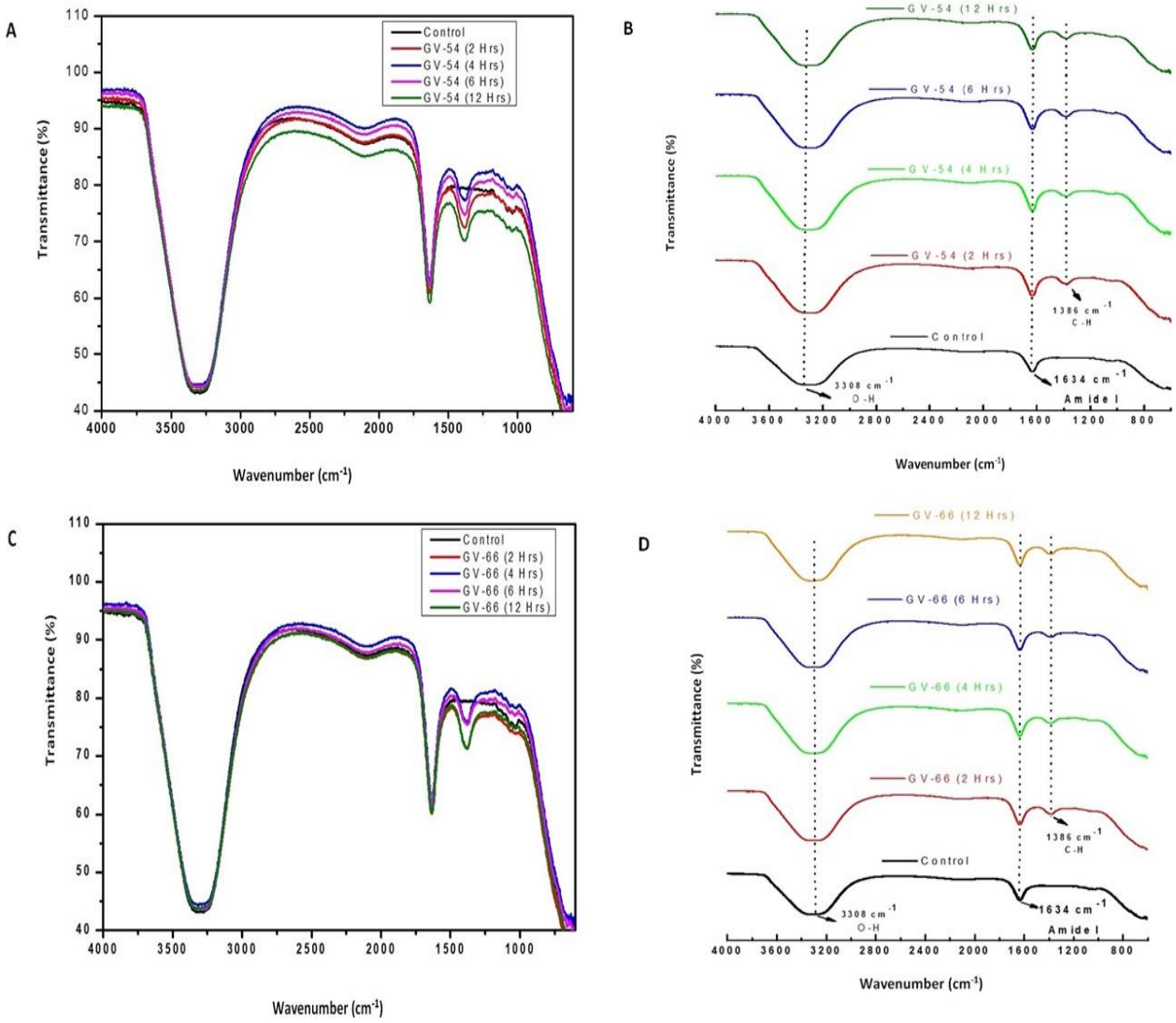

Fig. 1: FTIR analysis of lactose molecules hydrolysis by β-galactosidase (GV54 and GV66) at different time intervals of 2h, 4h, 6h, and 12h (A, B, C, and D). FTIR spectra of standard lactose (control), and lactose hydrolysis by β-galactosidase GV54 (A, B) and lactose hydrolysis by β-galactosidase GV66 (C, D).

Amide I group of β-galactosidase is uniformly bound to lactose at 1634 cm^{-1}. Based on the FTIR transmittance peaks observed for C-H group of glucose and galactose (at 1386 cm^{-1}), we could identify variations in the shape and strength of these peaks in the area 2000-800 cm^{-1}, compared to control (lactose solution without β-galactosidase enzyme). The spectra of β-galactosidase showed the distinctive protein peaks, which amply proved the enzyme's attachment to the surface of the lactose molecule. FTIR peaks of two samples showed that their bonds and vibrational modes are similar. Considering above characteristics, *L. plantarum* GV54 and *Lactiplantibacillus sp.* GV66 isolates significantly increased the production of β-galactosidase and hydrolyzed the lactose molecules.

DISCUSSION

The current investigation revealed that *Lactiplanti bacillus* isolates are a potential and promising source of the enzyme β-galactosidase. The isolate stabilized throughout a pH range of 4 to 9 and showed highest β-galactosidase activity at pH 7. β-galactosidase is a

tetrameric enzyme with four identical subunits and a molecular weight of ~116 kDa (22). β-galactosidase is used as a biosensor to estimate lactose present in milk and the production of lactose hydrolyzed milk for treatment of lactose intolerance (14, 15). The vibration modes of the O-H and -NH groups of the enzyme were identified to yield FTIR peaks between 3000 and 3500 cm^{-1}. Therefore, the O-H and -NH groups of β-galactosidase are responsible for the peaks seen at 3285 cm^{-1}. N-CH_3 stretching can be seen in the peaks at 2886 and 2942 cm^{-1}. Additionally, the strengthening of the peak at 444 cm^{-1} shows that β-galactosidase is uniformly bound to the nanoparticles (13). Along with its conjugation with the enzyme-nanoparticle, the FTIR spectra of free β-galactosidase from *Kluyveromyces lactis* and its immobilization on the zinc oxide nanoparticle were also investigated (13, 23).

The absorption of different carbohydrates in a specified spectral area was measured using FTIR spectra at 650-4000 cm^{-1}. The size and intensity of these peaks in the range 1200-900 cm^{-1} using the IR absorption peaks reported for glucose at 1033 cm^{-1}, fructose at 1063 cm^{-1}, and sucrose at 995 cm^{-1} (24). Earlier, Wang *et al.,* (25) investigated quantification of glucose, fructose, sucrose, and maltose in honey samples. All sugar mixes showed unique peak changes in the spectral "fingerprint" area between 1500 and 800 cm^{-1} (25). In another study, Ibrahim *et al.,* (17) investigated the O-H vibrations will extend between 3876 and 3005 cm^{-1}, whereas the CH vibrations will stretch up to 2061 cm^{-1}. Estimates for the C=O stretching range from 1849 to 1634 cm^{-1}. The peaks between 600 and 1500 cm^{-1} where the C-O and C-C group vibration modes exist and the characteristic banding pattern of carbohydrates is seen. On the other hand, the vibration groups CH and O-H are assigned to the bands from 2900 to 3450 cm^{-1}. Considering these characteristics we can promote *L. plantarum* GV54 and *Lactiplantibacillus* sp. GV66 as the most efficient lactose hydrolysers for dairy sector applications.

CONCLUSION

In this article, we used FTIR spectroscopy to evaluate the activity of β-galactosidase enzymes on lactose molecules in a safe, unblemished and practical manner. The precise and direct measurement of individual carbohydrates such as glucose and galactose in lactose solution hydrolyzed by β-galactosidase enzyme was successfully accomplished using FTIR spectrometry. Comparing FTIR analysis to traditional procedures, the time required was drastically decreased. These findings have the analytical power to follow the dynamics of *in vitro* enzymatic treatment of naturally occurring substrates rich in natural carbohydrate polymers.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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