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ORIGINAL ARTICLE

Induction Studies of a Novel α-Glucosidase Purified from *Lactobacillus fermentum* Grown on Resistant Starch

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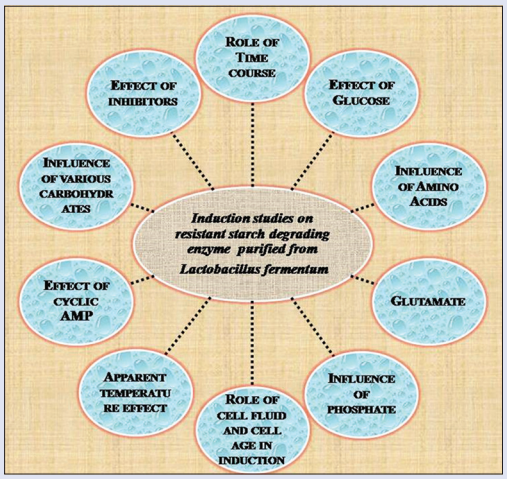
ABSTRACT

Aim: The aim of this study is to investigate the induction studies of resistant starch (RS)-degrading enzyme purified from *Lactobacillus fermentum*. **Background:** RS is one of the components of dietary fiber and considered as a good prebiotic. Earlier studies from our laboratory reporting *L. fermentum* to be the most efficient in degrading RS among several lactic acid bacteria tested. *L. fermentum* produced α-glucosidase when grown on RS, which was purified biochemically and characterized. **Results and Discussion:** The alpha-glucosidase was synthesized *de novo* and was repressed by glucose. Known protein synthesis inhibitors such as potassium cyanide, 2,4-dinitrophenol, and tetracycline were found to inhibit α-glucosidase synthesis. Cyclic adenosine 3′,5′-monophosphate was found to be a stimulator of α-glucosidase synthesis; however, it did not have any impact on the lag phase. Glutamate acted as an excellent nonrepressing carbon source. Maltooligosaccharides, dextrins, and soluble starch had varied influence on the induction of α-glucosidase synthesis. **Conclusion:** In the present communication, possible factors regulating α-glucosidase synthesis in *L. fermentum* were examined and discussed in terms of catabolite and apparent temperature-sensitive repression, culture age, induction, inhibitors, and various carbon sources.

Key words: Alpha-glucosidase, cyclic adenosine monophosphate, glucose, *Lactobacillus fermentum*, maltooligosaccharides, resistant starch

SUMMARY

- Many studies have been reported on dietary fibre and undigestible oligosaccharides with respect to their fermentation by lactic acid bacteria. However, very few studies were attempted to study the fermentation of RS by the above bacteria. In our earlier studies, we found that Lactobacillus fermentum is one such probiotic found to degrade RS and reported the purification and characterization of RS degrading enzyme,i.e., α-glucosidase from *L. fermentum* grown on RS. Since not many reports are available regarding the RS degrading enzyme, the main aim of the study is to check whether the α-glucosidase is produced by *L. fermentum* in logarithmic phase or stationary phase and also to examine the role of various compounds either in repression or induction of the α-glucosidase. The alpha-glucosidase was synthesised denovo and was repressed by glucose. Known protein synthesis inhibitors such as pottasium cyanide, 2,4-dintrophenol and tetracycline were found to inhibit α-glucosidase synthesis. Cyclic adenosine 3′,5′ monophosphate was found to be a stimulator of α-glucosidase synthesis, however it didn't have any impact on the lag phase. Glutamate acted as an excellent nonrepressing carbon source. Maltooligosaccharides, dextrins and soluble starch had varied influence on the induction of α-glucosidase synthesis.



Abbreviation used: RS: Resistant starch.

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INTRODUCTION

Plant cell polysaccharides, undigestible oligosaccharides and resistant starch are categorised under dietary fiber. Till the 1970s, starch was believed to be completely digestible in the small intestine unlike the dietary fiber components. Amylolytic enzymes such as alpha-amylase, pullulanase, and alpha-glucosidase are the enzymes involved in complete degradation of starch resulting in glucose, which in turn is utilized for various metabolic activities. Salivary and pancreatic alpha-amylases cleave the α-1,4-linkages in starch in a random fashion producing maltose, maltotriose, and branched dextrins consisting of α-1,6-linkages. Brush border intestinal microvilli are responsible for the secretion of sucrase, alpha-glucosidase, and isomaltase complex that cleaves the α-1,4, α-1,6-oligosaccharides into glucose culminating the process of starch digestion.[1] Extensive research in the 1990s from different laboratories across the globe unequivocally established the proof of RS (retrograded amylose) in several foods which were subjected to repeated heat and thaw treatments during cooking followed by storage of starch/starch-based food products. RS is defined as the starch that is not digested either by salivary or pancreatic amylase and gets fermented in the large intestine.[2] Probiotic microbiota is present in the human gut.[3,4] Many studies have been reported on dietary fiber

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and undigestible oligosaccharides with respect to their fermentation by lactic acid bacteria.[5] However, very few studies were attempted to study the fermentation of RS by the above bacteria. In our earlier studies, we found that *Lactobacillus fermentum* is one such probiotic found to degrade RS and reported the purification and characterization of RS-degrading enzyme, i.e., α-glucosidase from *L. fermentum* grown on RS.[6]

Since not many reports are available regarding the RS-degrading enzyme, the main aim of the study is to check whether the α-glucosidase is produced by *L. fermentum* in logarithmic phase or stationary phase and also to examine the role of various compounds either in repression or induction of the α-glucosidase.

Catabolite repression is the key factor involved in the global and specific gene-control mechanism.[7] In prokaryotes, various enzymes are synthesized through a combination of catabolite repression or transient suppression, wherein glucose acts as either inducer or suppressor in concentration-dependent manner, thus controlling the overall synthesis of hydrolytic enzymes.[8] It is a well-established fact that α-glucosidase is best produced in the presence of starch and dextrins rather than in the presence of glucose, wherein the enzyme synthesis generally decreases.[9] It is also aimed to check whether α-glucosidase synthesis is due to induction by RS or by low-molecular-weight oligosaccharides. In the present study, various factors such as effect of time, temperature, inhibitors, amino acids, different carbohydrates, cyclic adenosine monophosphate (AMP), culture age, and inorganic phosphate along with different concentrations of glucose were tested with respect to α-glucosidase induction in *L. fermentum* grown on RS.

MATERIALS AND METHODS

Chemicals and substrates

RS, dextrins, and glucose are taken at 0.2% (wt/vol). Glutamate (5mM), Aspartate (5mM), Succinate (15mM) and other carbon sources (8mM). Growth was calculated spectrophotometrically at 600 nm wavelength. Microscopic examination was used for determining the cell densities. RS were procured from HiMaize. Nalidixic acid, rifampicin, tetracycline-hydrochloride, and adenosine 3',5'-cyclic monophosphate were procured from Sigma Chemicals, Bangalore.

Bacterial strain and recovery of α-glucosidase

L. fermentum NCDC156 was obtained from National Collection of Dairy Cultures, NDRI, Karnal, India and further maintained on *Lactobacillus* MRS broth (HiMedia, Mumbai, India). Production of α-glucosidase was examined periodically by assaying the culture filtrates. During induction experiments, polysaccharides were autoclaved and mono- and oligosaccharides were sterilized by 0.45 μm pore size, 25 mm diameter (procured from MilliPore) membrane filters. Cells were harvested from the culture medium by centrifugation (4000 ×*g* for 20 min at 4°C). Various chemical, enzymatic, and mechanical methods were performed in combination or alone for solubilization of the membrane-bound enzyme. For disrupting the cells mechanically, ultrasonication (10 kHz) was performed for 15 min at 4°C followed by centrifugation (10,000 ×*g* for 20 min at 4°C). Cells were suspended in Mcilvaine buffer added with 0.1% mercaptoethanol and sonicated. Centrifugation was carried out for all the above fractions, and enzyme activities were measured in all the pellets and supernatants. Ultrasonication was carried out in ice for 3 min followed by centrifugation (3000 ×*g*, 20 min). The pellet was again subjected to the above procedure. Enzyme activity was measured in supernatant and was found to be membrane bound α-D-glucosidase activity.[10]

α-Glucosidase assay

The α-D-glucosidase enzyme activity was determined using p-nitroph enyl-α-D-glucopyranoside (pNPG) as the substrate. Substrate solution (5 mM of 900 μl) in 0.05 M potassium citrate buffer (pH 5.0) was mixed with the crude extract (100 μl) and incubated at 37°C ± 1°C for 20 min. Sodium carbonate (0.5 M of 100 μl)-saturated solution was added to stop the reaction. The amount of para-nitrophenol (PNP) released was determined by measuring the absorbance spectrophotometrically at 410 nm. One unit of enzyme activity is equivalent to 1 μ mole of PNP liberated from pNPG per min. Specific activity is defined in terms of units per mg protein.[10]

Effect of glucose on α-glucosidase synthesis

L. fermentum batch cultures, each containing glucose from 0 to 0.1% (wt/vol) and excess starch 0.2% (wt/vol), were examined for α-glucosidase activity in the culture supernatants. Separate experiments were conducted in which glucose (0–0.1%) was taken as sole carbon source and the culture density was determined till the glucose (carbon source) got exhausted.

Influence of amino acids on the induction of α-glucosidase synthesis

To test the influence of other carbon sources on α-glucosidase synthesis, *L. fermentum* was grown on tryptone as the carbon source both in the presence and absence of RS. To explore this possibility, cells were grown in media with excess RS along with a pool of amino acids such as serine, lysine glutamate, and aspartate in low concentrations of 2 mM respectively. Alpha-glucosidase levels were examined in the culture filtrates in the presence of aspartic acid and glutamic acid. Simultaneously, RS was added, and its effect on the enzyme activity was determined. Since glutamate and aspartate are metabolically related to glutamine and asparagine, the utilization of the latter two amino acids was also determined both in the presence and absence of RS for 8 h.

Induction of α-glucosidase synthesis by glutamate

Two identical *L. fermentum* cultures were grown on 10 mM concentration of glutamate as sole carbon source. To one of the culture filtrates, RS was added during the exponential phase of growth whereas the other culture filtrate served as control devoid of RS.

Role of time course

L. fermentum was cultivated aerobically in 2% RS at 30°C and culture filtrates were examined for α-glucosidase activity from 0th to 36 h with 4 h intervals to establish a progressive correlation between bacterial growth and α-glucosidase synthesis.

Effect of inhibitors

Tetracycline and rifampicin (100 μg per ml), KCN, and 2,4-dinitrophenol (5 mM) were added to the culture filtrates at regular time intervals of 24 h, whereas nalidixic acid was added at a concentration 100 μg per ml at time intervals of 4 h during 16–28 h.

Apparent temperature effect on α-glucosidase synthesis

Experiments were conducted to determine the effect of temperature on α-glucosidase synthesis and growth of the cultures. It was observed that 30°C was the optimum temperature for growth and α-glucosidase synthesis. Thereafter, the cultures were shifted from 30°C to 50°C and 50°C–30°C at different growth phases to examine the effect of temperature shift on α-glucosidase synthesis.

Influence of various carbohydrates

L. fermentum was grown on medium taking RS as the sole carbon source. Individual carbohydrates: monosaccharides such as glucose, galactose, xylose, and rhamnose; maltooligosaccharides, namely maltoheptaose, maltopentaose, and maltotriose; disaccharides such as lactose, maltose, and sucrose; trisaccharides, namely raffinose; and polysaccharides such as RS, dextrin, inulin, and starch were added (0.5% wt/vol) to 48 h grown cultures at 30°C to examine their effect on α-glucosidase synthesis after 3 days.

Effect of cyclic adenosine monophosphate

Effect of 5 mM cyclic AMP was tested on the 2nd, 3rd, and 4th days of culture filtrates of *L. fermentum* grown on RS with respect to α-glucosidase synthesis.

Role of cell fluid and cell age in induction of α-glucosidase synthesis

Cells of different age were transferred to culture fluids, which previously supported the growth of *L. fermentum,* and α-glucosidase synthesis was determined separately in the presence of 0.5% (wt/vol) RS and glucose.

Influence of phosphate

L. fermentum cultures were grown on minimal maltose media containing phosphate buffer in the concentration range of 12–300 mM. To explore the effect of phosphate buffer on α-glucosidase synthesis, cells were harvested from late exponential phase, washed in physiological saline, and resuspended in prewarmed medium. Experiments were done in 50 ml conical flask at 37°C in shaking water bath.[11] Harvested cells were resuspended in media which contained disodium hydrogen phosphate (10 mM), potassium dihydrogen phosphate (10 mM), glucose 6-phosphate (3 mM), glucose 1-phosphate (3 mM), and AMP (3 mM) at pH 7.0. Further, washed cells were treated with different concentrations of phosphate buffer added with chloramphenicol (20 μg/ml) and rifampicin (1 μg/ml).

Statistical analysis

All the experiments were performed in triplicates and the values were represented as mean values ± standard deviation.

RESULTS

Repression of α-glucosidase synthesis by glucose

Alpha-glucosidase production was significantly greater in the presence of RS than glucose. Maximum α-glucosidase activity obtained was 54.00 (±0.02) U/ml, indicating that production of enzyme was in response to the availability of RS. Due to the severe nature of the extracellular environment, the ongoing enzyme synthesis may be coupled with an enzyme inactivation, which is demonstrated by the plateau formation. This possibility is investigated by subjecting a sample with α-glucosidase activity (cell-free supernatant) to 60 h prolonged incubation at 85°C at pH 3. The supernatant retained most of its initial activity (96%). This result signifies that the plateau formation is due to the termination of enzyme production but not due to the enzyme inactivation.

During the growth of the bacteria on RS, the termination in α-glucosidase production might be due to the accumulation of glucose as a repressing metabolite for α-glucosidase synthesis. Glucose omission resulted in an immediate synthesis of α-glucosidase. There was an appearance of α-glucosidase in the culture supernatants grown on series of decreasing concentrations of glucose corresponding to the cell densities examined for glucose exhaustion [Figure 1]. Following cell yields were obtained: 0.0125%, 1.93 × 10^9 cells per ml; 0.025%, 3.6 × 10^9 cells per ml; and

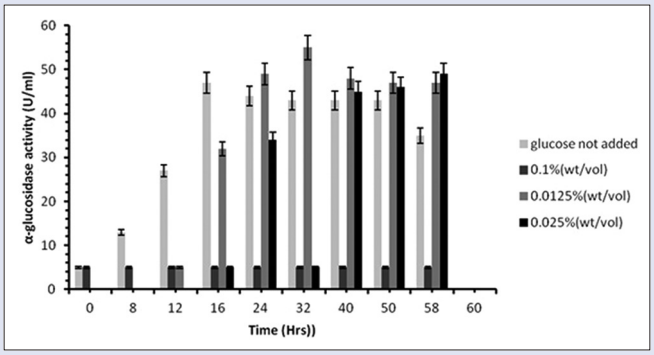

Figure 1: Repression of α-glucosidase by glucose. Cells were grown in separate batches containing starch along with 0.1%, 0.0125%, and 0.025% glucose and without glucose

0.05%, 6.3 × 10^9 cells per ml. There was no effect on the cell growth in the presence of limiting glucose and excess starch in spite of having differences in growth on starch and glucose. No α-glucosidase synthesis was observed both in the conditions of glucose starvation as well as resupplementation of glucose.

Regulation of α-glucosidase synthesis by amino acids

A positive correlation between the α-glucosidase levels produced in the presence and absence of RS along with other carbon sources was observed [Figure 2]. Comparatively, aspartate was a more repressing carbon source than glutamate. Although the two amino acids exhibited differences in α-glucosidase levels, cells exhibited equal efficiency in the utilization of the amino acids along with either of the carbon source. Asparagine repressed the α-glucosidase levels (no enzyme activity found) whereas glutamine nonrepressed (13.90 U/ml ± 0.02). Among the tricarboxylic acid cycle intermediates used as the carbon sources, growth was supported by only succinate (2.10 U/ml ± 0.01). Growth was not observed upon adding isocitrate, malate, fumarate, and oxaloacetate (data not shown). Either in the presence or absence of RS, there was an intermediate effect on α-glucosidase synthesis by glucose or succinate during generation times of 8 h or 36 h. Thus, α-glucosidase synthesis is responsive to the utilization of sole carbon source for growth irrespective of the cell growth rate.

Role of glutamate in induction of α-glucosidase synthesis

In *L. fermentum*, aspartate and glutamate get converted to tricarboxylic acid intermediates and other amino acids. High levels of α-glucosidase observed in the presence of glutamate indicated it to be a nonrepressing source of carbon on α-glucosidase synthesis. Hence, a further examination of the utility of glutamate as a chief carbon source can enable evaluation of kinetics of α-glucosidase synthesis by addition of RS. On addition of RS, maximum α-glucosidase activity (12.80 U/ml ± 0.01) was detected within one cell generation, whereas there were low traces of α-glucosidase activity in the supernatant of culture growth without RS 0.12 ± 0.02 U/ml [Figure 3].

Time course of α-glucosidase synthesis in growing cultures of *Lactobacillus fermentum*

Growth profile exhibited a logarithmic phase for about 4 h followed by a lag phase for 6–8 h, in which the bacterial cells doubled thereafter reaching a stationary phase. The cell concentration was almost constant

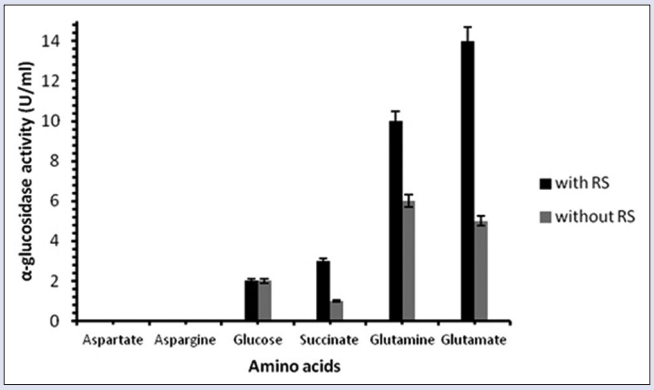

Figure 2: *Lactobacillus fermentum* cultures were grown in the presence of various carbon sources both in the presence and absence of resistant starch

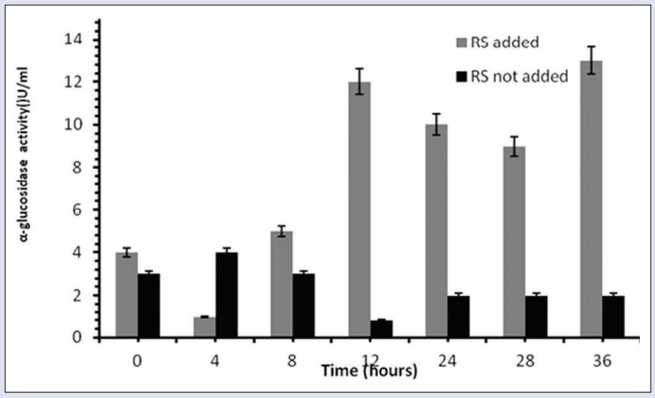

Figure 3: In the presence of glutamate, α-glucosidase induction was determined by addition and nonaddition of resistant starch to the medium

in stationary phase for about 24 h and then decreased gradually. α-Glucosidase synthesis occurred at 48 h when *L. fermentum* was cultivated in the standard medium at 30°C; however, maximum accumulation was observed by the end of the experimental procedure, i.e., 96–120 h (data not shown).

Impact of inhibitors on α-glucosidase synthesis

Tetracycline and rifampicin hindered the α-glucosidase activity within 2 h, unlike the effect of KCN and 2,4-dinitrophenol which took more time, i.e., 1 day [Table 1]. The reason may be due to a deficiency in the minimum availability of adenosine 3'-triphosphate during α-glucosidase synthesis. Cell lysis occurred upon addition of these antibiotics and inhibitors to the logarithmic phase. Nalidixic acid which is an inhibitor of deoxyribonucleic acid synthesis exhibited a different effect on α-glucosidase synthesis than the other inhibitors; at 16–20 h, there was a complete suppression of enzyme activity and continued thereafter.

Effect of temperature shift on α-glucosidase synthesis

The temperature dependence of α-glucosidase synthesis in *L. fermentum* is shown in Figure 4. There was an abrupt increase in the cell mass during the transfer from 30°C to 50°C, but there were no traces of α-glucosidase levels until 72 h (day 3) in spite of growing the culture prior at 30°C [Figure 4a]. In the case of cultures shifted from 50°C to 30°C, cell lysis was observed with no significant α-glucosidase activity in cells except for those cultures in which α-glucosidase secretion has started in the beginning remained to synthesize α-glucosidase even after the shift in the temperature [Figure 4b].

Induction of α-glucosidase synthesis by various carbohydrates

Alpha-glucosidase synthesis was induced in the presence of polysaccharides such as dextrins and RS. Maltooligosaccharides, namely Maltoheptaose, Maltohexaose, Maltopentaose, Maltohexaose, and Maltotetraose, act as substrates for α-glucosidase synthesis in *L. fermentum* [Table 2].

Repression of α-glucosidase synthesis by catabolite

The formation of α-glucosidase in *L. fermentum* was immediately suppressed upon addition of 0.5% acetates, succinate, glycerol, and glucose. There was no observation of enzyme synthesis in noninduced cultures even upon addition of glucose and starch. Thus, it is clear that α-glucosidase synthesis is sensitive to catabolite repression. About 40%–60% of the α-glucosidase synthesis was stimulated upon addition

Table 1: Effect of inhibitors on α-glucosidase synthesis in *Lactobacillus fermentum* cultures grown on resistant starch

	Culture days					
	0	1	2	3	4	5
Control	0	9.00±0.02	12.00±0.01	15.00±0.02	21.00±0.01	25.00±0.01
Tetracycline	0	1.00±0.11	4.00±0.11	4.00±0.11	4.00±0.011	4.00±0.011
Rifampicin	0	5.00±0.02	9.00±0.07	2.00±0.06	2.00±0.06	2.00±0.05
2,4 DNP	0	6.00±0.01	9.00±0.01	8.00±0.02	7.00±0.02	7.00±0.02
KCN	0	5.00±0.02	9.00±0.02	15.00±0.02	15.00±0.01	15.00±0.01

Cultures were grown on standard medium in tubes at 30°C. Inhibitors added were rifampicin and nalidixic acid at concentration of 100 μg/ml, KCN, and 2,4 dinitrophenol at concentration of 5 mM respectively

Table 2: Influence of various carbohydrates on α-glucosidase activity

Carbohydrates*	α-glucosidase activity (U/ml)
Monosaccharides	
D-Glucose	0
D-Galactose	34.00±0.01
D-Xylose	32.00±0.03
L-Rhamnose	12.00±0.03
D-fructose	19.00±0.03
D-Mannose	23.00±0.01
Inositol	19.00±0.02
D-Arabinose	12.00±0.04
Disaccharides	
Lactose	103.00±0.01
Maltose	92.00±0.04
Sucrose	38.00±0.02
Trisaccharides	
Raffinose	94.00±0.03
Maltooligosaccharides	
Maltoheptaose	78.00±0.04
Maltohexaose	69.00±0.01
Maltopentaose	126.00±0.03
Maltohexaose	116.00±0.02
Maltotetraose	111.00±0.04
Polysaccharides	
RS	60.00±0.04
Dextrin	109.00±0.04
Starch	130.00±0.01
Inulin	0

*0.5% carbohydrates added. RS: Resistant starch

Table 3: *Lactobacillus fermentum* culture filtrates at various ages were centrifuged and the cells were washed with 0.5 M Tris buffer (pH 7.2) and added to fresh media to measure the growth at 600 nm

Culture filtrates of *Lactobacillus fermentum*					
Number of days i.e., age of the cells	Alpha-glucosidase synthesis (U/ml)				
	0	1	2	3	4
1	0.40±0.03	14.00±0.01	26.00±0.02	32.00±0.02	16.00±0.03
2	0.90±0.01	19.00±0.02	28.00±0.03	34.00±0.02	18.00±0.02
3	0.80±0.02	18.00±0.03	34.00±0.02	27.00±0.03	13.00±0.01
RS (0.5%)			53.00±0.01		
Glucose (0.5%)			13.00±0.03		

RS: Resistant starch

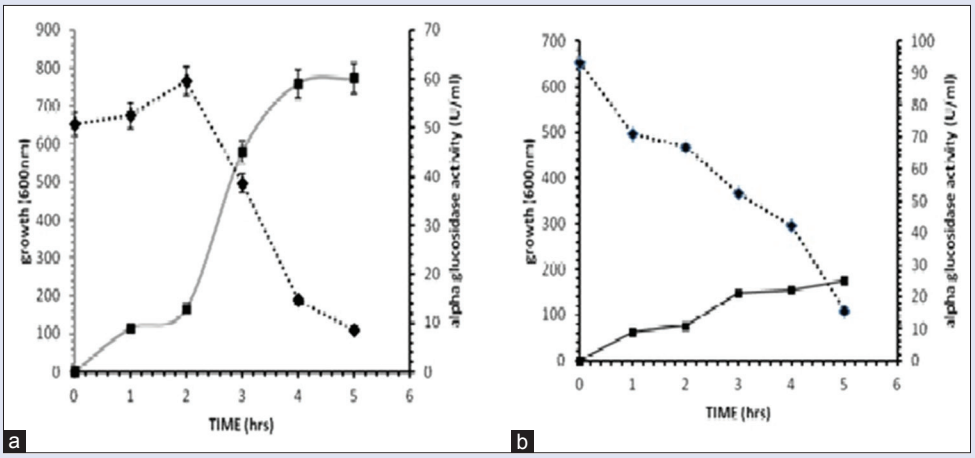

Figure 4: Induction of α-glucosidase synthesis by temperature shift *in L. fermentum* grown on resistant starch. Zero-day cultures were transferred from (a) 30°C–50°C (b) and 50°C–30°C

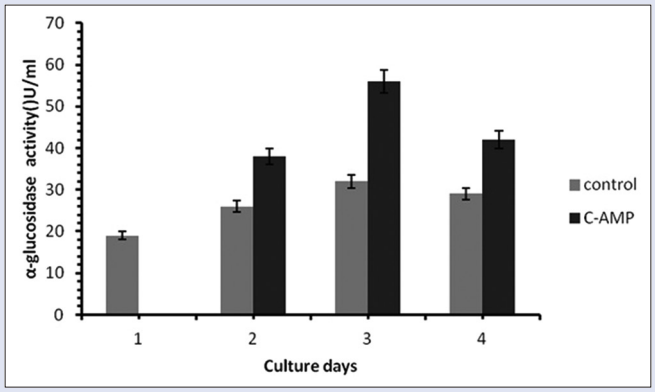

Figure 5: Induction of α-glucosidase synthesis by cyclic adenosine monophosphate *in Lactobacillus fermentum* grown on resistant starch. Cultures were grown on standard medium at 30°C. Cyclic adenosine monophosphate was added at days 2, 3, and 4

of cyclic AMP [Figure 5]; however, there was neither shortening of lag phase, nor alleviation effect of glucose.

Induction of α-glucosidase synthesis by cell age and cell fluid age

A combination of 2- and 3-day-old fluid culture fluid was found to be the best for α-glucosidase synthesis. The addition of 0.5% RS (wt/vol) to this combination increases α-glucosidase formation, whereas adding 0.5% glucose (wt/vol) caused repression of the enzyme synthesis confirming catabolite repression. Thus, the data clearly indicate the effect of culture fluid on α-glucosidase synthesis [Table 3].

Effect of phosphate on α-glucosidase synthesis

At pH 7.0, cultures remained normal, and at pH 4.5 (12 mM) and pH 5 (300 mM), the cultures were taken from exponential growth phase. α-Glucosidase levels were more at initial phosphate buffer concentrations of 12 mM and were gradually reduced to one-tenth of initial level [Figure 6]. Maltose utilization was impaired at relatively low concentrations of phosphate. In the presence of inorganic phosphates, α-glucosidase levels were high as compared with that of glucose phosphates. There was no effect on α-glucosidase synthesis by ATP and AMP, and the cells were identical to that of the culture devoid of phosphate. The failure of adenine nucleotide to stimulate α-glucosidase synthesis may be due to cellular impermeability of these molecules.[8] Rifampicin and chloramphenicol mainly hinder the incorporation of uracil into RNA and leucine into protein, respectively.[12] Among these two antibiotics, chloramphenicol decreased the α-glucosidase synthesis more than that of rifampicin, indicating that phosphate did not merely affect the release of preformed enzyme. It may be that phosphate may increase messenger RNA stability by inhibiting RNase activity, or alternatively, it may affect the cytoplasmic membrane such that membrane-bound ribosomes are better adapted for protein translation.[8]

DISCUSSION

The enzyme reported here is a membrane-bound α-glucosidase. It is an exoenzyme, hydrolyzing the glucose from the nonreducing ends of either starch or dextrins. Analysis of culture filtrates of *L. fermentum*

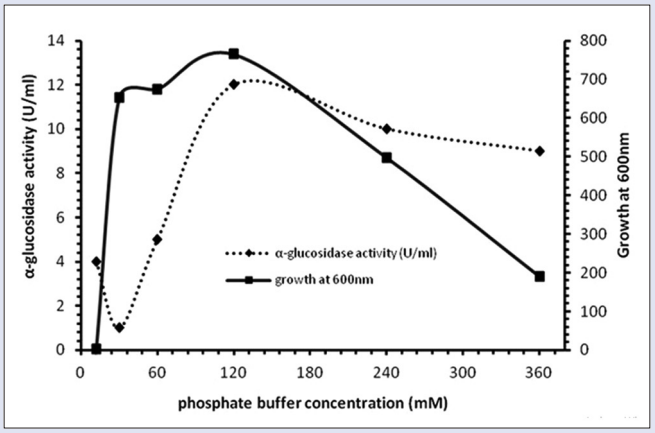

Figure 6: α-Glucosidase yield and cell yield during the growth of *Lactobacillus fermentum* on different concentrations of phosphate buffer

grown on RS exhibited α-glucosidase synthesis. Levels of α-glucosidase varied in the culture supernatants of *L. fermentum* with respect to the nature of carbon source added. Substrate induction of α-glucosidase synthesis by RS ceased relatively early under all the conditions examined. Addition of glucose caused repression of α-glucosidase synthesis, which might be due to the pooling up of hydrolysis products of RS, i.e., glucose. Similar effects were seen in *Sulfolobus solfataricus* where α-glucosidase is repressed by hydrolyzed products formed by the action of α-amylase.[13] Induction of α-glucosidase synthesis by RS and repression by glucose indicate that the α-glucosidase from *L. fermentum* can be subjected to multiple forms of regulation. In the presence of nonrepressing carbon source such as glutamate, the induction of α-glucosidase synthesis by the addition of substrate becomes apparent. The presence of carbon sources as repressing or nonrepressing for α-glucosidase synthesis further explains the similarity between catabolite repression as observed in *L. fermentum*.[8]

Maximal growth rates were shown in the presence of aspartate and glutamate with remarkable differences in α-glucosidase synthesis. There were reports contrast to the present study wherein a positive correlation was made between cell growth and α-glucosidase synthesis.[14] Differential production in enzyme in response to aspartate and glutamate was seen previously.[15] Glucose repression of α-glucosidase synthesis explains the catabolic control over this enzyme. In the second type of mechanism, the catabolic effect on the enzyme is due to other carbon sources, which are not dependent on the availability of starch *in situ*. The above two forms of regulation of α-glucosidase synthesis may constitute a generalized response to carbon source.[16]

There is likely induction of alpha-amylase when there was only cell membrane-bound α-glucosidase synthesis found throughout the growth phase, lag phase, and lysis. The above analysis along with the observations seen during the addition of inhibitors suggested that α-glucosidase synthesis in *L. fermentum* is a *de novo* protein synthesis which is in agreement with the existing literature.[17,18] Temperature-sensitive repression, induction, culture age, and catabolite repression were the key factors controlling α-glucosidase synthesis.[19] Cyclic AMP had shown a weak but significant effect on α-glucosidase synthesis, but neither it could shorten the lag phase nor overcome glucose repression. This observation is perhaps due to the poor uptake of cyclic AMP by *L. fermentum* or any indirect effect of cyclic AMP independent of catabolite repression. At higher temperatures (50°C), α-glucosidase synthesis exhibited a catabolite repression; later by the addition of inducers, there was a withdrawal in repression. This may be due to a decrease in the low-molecular-weight metabolites present in intracellular level. RS, being polysaccharide, is apparently too large to enter the cell and thus itself cannot be an inducer. Low-molecular-weight maltooligosaccharides or maltodextrins present in the RS that were formed by the effect of α-glucosidase may be the possible inducers.

Maltooligosaccharides were found to be potent inducers of α-glucosidase synthesis in *L. fermentum* than RS [Table 2]. According to literature, amylases from some bacteria do not act much on molecular weights less than maltopentose.[20] Hence, in this context, results are in tune with few reports where maltotriose to maltohexose was inducing the α-glucosidase [Table 2]. Inducibility of α-glucosidase occurs in "constitutive" strains; however, there were few strains which do not undergo catabolite repression but still produce α-glucosidase when grown on glucose.[21] Observations on induction of α-glucosidase synthesis due to change in the fluid parameters are summarized in Table 3. Thus, either an inducible or depressible elements are responsible for the involvement of cell age and culture fluid in α-glucosidase synthesis. The effect of phosphate on α-glucosidase synthesis was confirmed by the washed cell experiments, and low enzyme synthesis was perhaps not due to phosphate starvation. In the presence of rifampicin, phosphate had a stimulatory effect confirming its involvement in protein synthesis level. The reason may be that RNase activity is inhibited as the phosphate increases the stability of messenger RNA.[22] The present study dealt with the possible factors regulating alpha-glucosidase synthesis in *L. fermentum*. Glucose repression was reported and is concentration dependent. Potassium cyanide, 2,4-dintropheol, and tetracycline inhibited the α-glucosidase synthesis at different time intervals. Cyclic adenosine 3',5'-monophosphate was found to be a stimulator of α-glucosidase synthesis but did not have any effect on the lag phase. Glutamate acted as an excellent nonrepressing carbon source. Maltooligosaccharides, dextrins, and soluble starch had varied influence on the induction of α-glucosidase synthesis.

CONCLUSION

In the current study, the induction studies pertaining to the RS-degrading enzyme from *L. fermentum* were carried out. The nature of the enzyme was studied under different conditions, parameters, and inhibitors. Further, the cloning of the current enzyme is under progress.

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Conflicts of interest

There are no conflicts of interest.

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