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SOME PROPERTIES OF β-GALACTOSIDASE PRODUCED FROM *Bifidobacterium bifidum* AND ITS USE IN MILK LACTOSE HYDROLYSIS

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ABSTRACT: Bifidobacteria, which have beneficial health effects, are known to have relatively high levels of β–galactosidase. Therefore, these results revealed that: enzyme productivity reached its maximum after 48 hours of incubation. 37°C and pH 4.5 were found as the best environmental conditions for enzyme biosynthesis. 5% lactose in the production media induced the highest synthesis of enzyme. 30°C was found as the optimum temperature for enzyme activity. The enzyme kept all activity up to 40°C and lost only 15% from its maximum activity at 50°C. Residual enzyme activity declined sharply when the enzyme was kept above 50°C, indicating that this enzyme isn't thermostable one. pH 5.0 was found as optimum for enzyme activity. β–Galactosidase was kept all activity in the pH range 5.0 to 6.0 and lost 5% and 7% at pH 4.5 and 6.5, respectively. Outside this pH range, the enzyme lost most of its activity. Mg^{+2}, Na^{+}, Ca^{+2} and Mn^{+2} activated enzyme protein as well as enzyme activity. Other metal ions such as Zn^{+2} and Fe^{+3} and Fe^{+2} inhibited the enzyme activity. Hydrolysis of milk lactose existed with the increasing of β–galactosidase concentration. Also, decreasing milk lactose content was pronounced with over production of titratable acidity. So, the addition of this enzyme or/and probiotics bacteria producing enzyme to dairy products led to production of dairy products with lowest lactose intolerance.

Keywords: *Bifidobacterium bifidum*, β–galactosidase, enzyme production, properties, lactose content, titratable acidity.

INTRODUCTION

Probiotics microorganisms including lactic acid bacteria and bifidobacteria, are very important in the treatment of a wide range of human disorders including lactose intolerance, diarrhea, food allergies, intestinal infection, constipation, gastroenterities, hepatic, encephalopatny, flatulence, colitis, gastric acidity, high blood cholesterol and cancer (Badawi & El-Sonbaty, 1997; Dechter & Hoover, 1998; Godward *et al.*, 2000 and Gooda *et al.*, 2002). These strains produce natural antibiotics and organic acids (Rasic, 1983 and Gooda *et al.*, 2002). Also, these organisms have anticarcinogenic effect that can prevent the conversion of procarcinogenic into carcinogenic due to the content of their enzymes such as β–galactosidase, nitroreductase and azoreductase (Reddy *et al.*, 1983; Goldin and Gorbach, 1984 and Gooda *et al.*, 2002).

More recent, world-wide interest in nutriceutical foods, especially in prebiotic compounds (bifidus growth factors) and probiotic cultures has led to a rapid incorporation of bifidobacteria for commercial use as directly adjuncts such as cottage cheese, sour cream, lite ice cream and orange juice as well as fermented milks (Dechter & Hoover, 1998). Maintenance of normal intestinal microflora, antimicrobial activity, synthesis of vitamins and increased calcium absorption are some of the benefits attributed to bifidobacteria (Hughes & Hoover, 1991 and Dechter & Hoover, 1998). Therefore, the uses of bifidobacteria of human origin are most popular and thought to be necessary (Ishibashi & Shimamura, 1993 and Dechter & Hoover, 1998).

The hydrolysis of the β-galactosidic bonds between galactose and glucose in the lactose molecule by β-galactosidase can be used as a method for increasing digestibility of lactose-containing products and may even improve functional properties of some dairy products. Decreasing the level of lactose in whey and other milk products can increase sweetness, decrease the grittiness in ice cream caused by crystallized lactose, and can improve the utilization level of high protein supplements containing milk (Ismail *et al.*, 1997 and Shady & Abdel-Razik, 1997). All these problems can be solved by enzymatic hydrolysis of lactose

in milk products and whey by using β–galactosidase (EC 3.2.1.23). This enzyme has been isolated from several microbial sources. But, limited information about the production of this enzyme from a health benefits of bifidobacteria were reported (Hussain *et al.*, 1995; Shady & Abdel-Razik, 1997 and Decher & Hoover, 1998).

Therefore, the present study deals with the production and properties of β–galactosidase from *Bifidobacterium bifidum* and use the enzyme for hydrolysis the milk lactose for improving the nutritional and healthy benefits of milk and other dairy products.

MATERIALS AND METHODS

Microorganisms and fermentation technique:

Bifidobacterium bifidum was obtained from DRIVAC Lactic Culture CHR Hansen's Laboratories Copenhagen, Denmark.

The strains used were cultivated weekly in the Lactobacilli MRS both (Difco Laboratories, Detroit, MI) supplemented with 0.05% (w/v) L-cysteine, 0.075% Bacto-Agar (Difco), 0.02% (w/v) Na_2CO_3 and 0.01% (w/v) $CaCl_2.2H_2O$. Cultures were incubated aerobically for 15 h at 37°C without agitation. For inoculum preparation, cells of cultures grown 48 h in modified MRS broth were harvested by centrifugation at 8000 rpm, suspended in sterile 5% NFMS (non-fat milk solids) plus 8% sucrose and 1.5% gelatin and then stored at 5-7°C prior to use (Collins and Hall, 1984).

Cultivation for 72 hours was carried out in cotton-plugged Erlenmeyer flask (250 ml) containing 45 ml of MRS broth and 5 ml of the above cell suspension. The cultures were incubated at 37°C. After incubation for 72 hours, the cells of the culture fermentation were autolyzed with chloroform (2% w/v) at 37°C for 10 min. The broken cells were removed from the assay mixture by centrifugation at 15 000 rpm for 30 min at 4°C. The cell free extracts were used as a source of enzyme (Shady and Abdel-Razik, 1997).

Media:

MRS medium was used, which has the following composition (g/Liter): peptone from casein, 10.0; meat extract, 8.0; yeast extract, 4.0; glucose, 20.0; dipotassium hydrogen phosphate, 2.0; sodium acetate, 5.0; magnesium sulphate, 0.2;

manganese sulphate, 0.4; agar agar (not present in MRS broth), 14.0 (Brinchmann *et al.*, 1983).

Enzyme assay:

Enzymatic activities were determined by measuring the amount of glucose released from hydrolysis of lactose according to the method described by Fantes and Roberts (1973) with some modification by Shady and Abdel-Razik (1997). Each reaction mixture contained 1.5 ml of phosphate buffer (pH 6.8) 0.03 ml $MgCl_2$ (0.01 M), 0.3 ml lactose (5%), 0.3 ml of enzyme source was added and completed by distilled water up to 3.0 ml. The mixture was incubated at 37°C. After 10 min, the reaction was stopped by boiling enzyme reaction mixture for 10 min. Reducing sugars (as glucose) were determined by colorimetric estimation of lactose and its hydrolytic products (Nickerson *et al.*, 1976). One enzyme unit is defined as the amount of enzyme that released one mg glucose /min under the assay conditions.

Thermal and pH stability of enzyme:

Thermal β–galactosidase stability was determined by incubating the enzyme solution with buffer at the optimum pH without substrate for one hour at 20-80°C. The residual enzyme activity was examined under the optimal conditions.

pH stability of enzyme was assayed by determination the residual activity after incubation the enzyme solution in an appropriate buffer (pH 4.0-8.5) at optimum temperature for one hour. Then, the residual activity of these preincubated samples was measured.

Lactose content:

All samples of pasteurized milk, before and after enzyme treatments examined for lactose content according to Nickerson *et al.* (1976).

Titratable acidity:

It was determined as lactic acid percentage using the method described by Arbuckle (1986).

Hydrolysis rate %:

It was calculated as released glucose against lactose found in different sources x 100 (glucose/lactose x 100).

RESULTS AND DISCUSSION

The use of bifidobacteria in cultured or culture-containing milks received greater attention for their healthy and therapeutic action

as well as its sufficient or relatively high levels of β–galactosidase which produce low-lactose dairy products. Therefore, the results illustrated in Fig. (1) show that *Bifidobacterium bifidum* produced β–galactosidase at the beginning of incubation period and increased gradually with the extension of incubation time up to 48 hours, then, enzyme activity decreased slightly. This means that this microorganism can ferment dairy products containing lactose directly after manufacturing. The maximum productivity of this enzyme was attained after 48 hours thereafter decrease with slight amount. Similar results were reported by El-Sawah *et al.* (1991) and Shady & Abdel-Razik (1997).

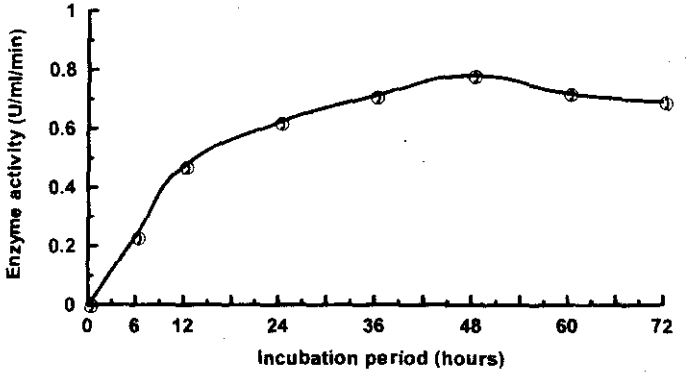

Fig. (1): Effect of incubation period on β–galactosidase production.

Results illustrated in Fig. (2) show the effect of incubation temperature of *B. bifidum* β–galactosidase activity. The results showed that enzyme biosynthesis was recorded at refrigerator temperature (5-7°C) and highly increased up to 37°C, thereafter decreased greatly. 35-37°C were found as the optimum temperature range for enzyme biosynthesis. The results in general showed that *B. bifidum* produced this enzyme constitutively. Also, it could be observed from these results that this bacterium may produce this enzyme at different storage temperatures of many dairy products such storage at refrigerator or/and at room temperature. Shady and Abdel-Razik (1997) reported that 28-32°C were found as the optimum temperature for enzyme production

by *Kluyveromyces fragilis*. Dechter and Hoover (1998) found that bifidobacteria produced β–galactosidase at frozen storage.

Effect of initial pH:

From data illustrated in Fig. (3), it was noticed that, enzyme biosynthesis was increased up to pH 4.5, which recorded highest enzyme productivity (1.99 Units/ml) and, thereafter, decreased sharply. This means that, the isoelectric point of protein precipitation (pH, 4.6) was the best and favoured pH for enzyme synthesis. These results are in harmony with those reported by Fenton (1982) and Shady *et al.* (1997). However, Dawoud *et al.* (1997a) found that pH 3.0 was the optimum pH for *Kluyveromyces fragilis* β–galactosidase production.

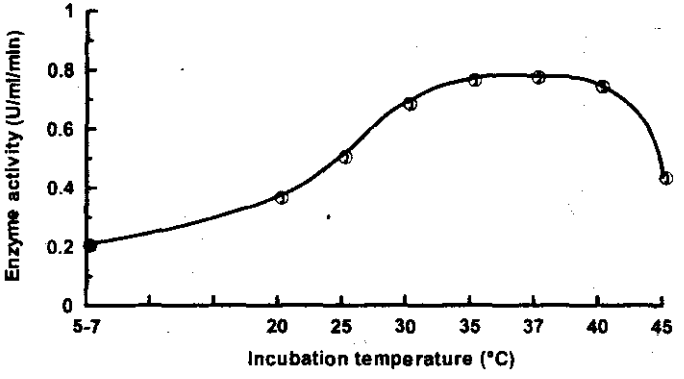

Fig. (2): Effect of incubation temperature on β–galactosidase production.

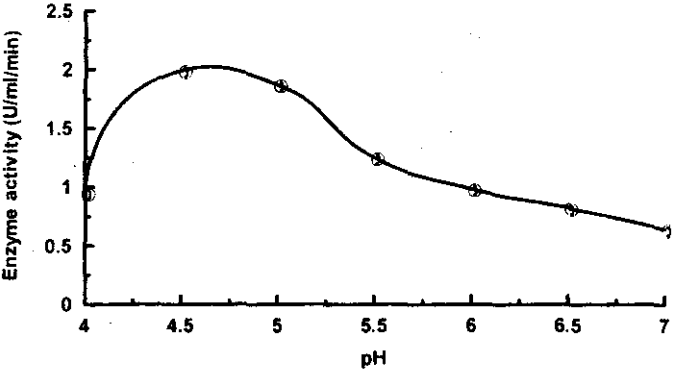

Fig. (3): Effect of initial pH on β–galactosidase production.

Effect of different carbon sources on enzyme production:

The effect of different carbon sources on the fermentation process were studied and the results of Fig. (4) showed that, using other carbon sources instead of glucose in the fermentation media supported and enhanced the biosynthesis of enzyme such as disaccharide, *i.e.*, lactose, maltose and sucrose. Other ones such as cellobiose, and fructose were repressed the enzyme formation. Lactose was found as the best carbon and energy source used for enzyme production. Maltose and sucrose were found as the best inducers for enzyme production but were found in the second order. This means that, the type of sugar in the fermentation media was affecting greatly on enzyme production. Dawoud *et al.* (1997a) reported similar results.

Effect of lactose concentration on enzyme production:

In this experiment, the fermentation media was fortified by different concentrations of lactose as the best source of carbon and energy. The results in Fig. (5) show clearly that the biosynthesis of enzyme was affected with the different concentrations of lactose. The enzyme productivity increased greatly up to 5% and then decreased sharply. The increasing of lactose concentration of the fermentation media above to 5% decreased the enzyme biosynthesis, which repressed it greatly. These findings are similar to those obtained by Shady *et al.* (1997).

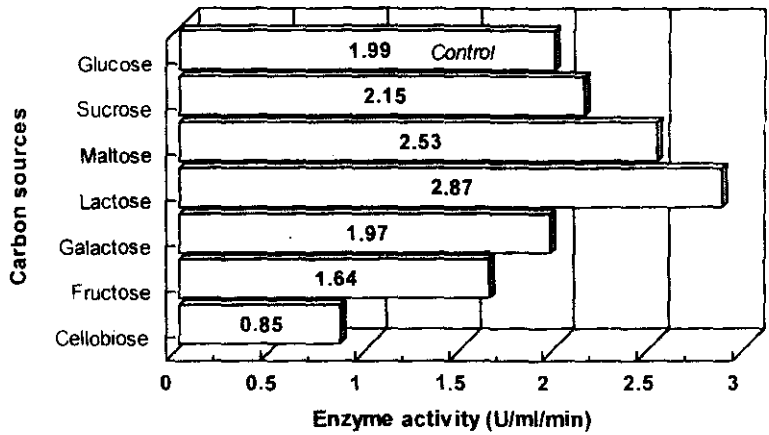

Fig. (4): Effect of different carbon sources on β–galactosidase production.

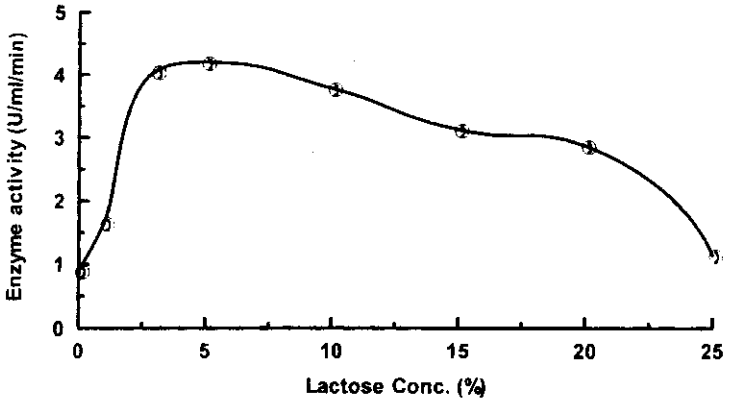

Fig. (5): Effect of lactose concentrations on β–galactosidase production.

Enzyme properties:

The influence of temperature on β-galactosidase activity was examined and the obtained results are illustrated in Fig. (6). The maximum enzyme activity was found to be at 30°C. Above or below this temperature degree, enzyme activity decreased sharply. Also, these results showed that the optimum temperature of this enzyme was in the temperature range used for ripening of dairy products. So, this enzyme is of practical importance for lactose hydrolysis in milk and dairy products being treated around this temperature. Similar results were obtained by Hussain *et al.* (1995). However, Shady & Abdel-Razik (1997) and Shady *et al.* (1997) found that 40°C is the optimum temperature for enzyme activity.

Thermal inactivation of enzyme activity:

The enzyme was incubated at different temperatures ranged between 20 to 80°C for one hour. The results (Fig. 7) indicated that the enzyme kept all activity up to 40°C, thereafter, enzyme activity decreased. The increasing of temperature above 40°C, decreased greatly the enzyme activity, which reached it's minimum activity (19%) at 80°C. This means that pasteurization temperature of milk lost maximum activity of this enzyme. Therefore, this enzyme isn't thermostable one. Similar results were recorded by Ismail *et al.* (1997).

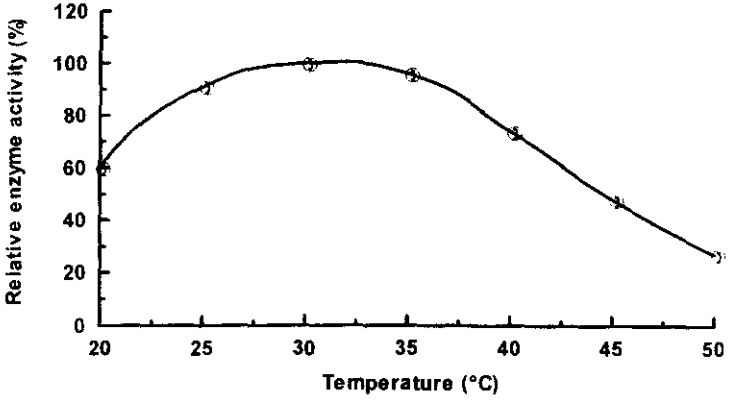

Fig. (6): Temperature optima for *B. bifidum* β–galactosidase activity.

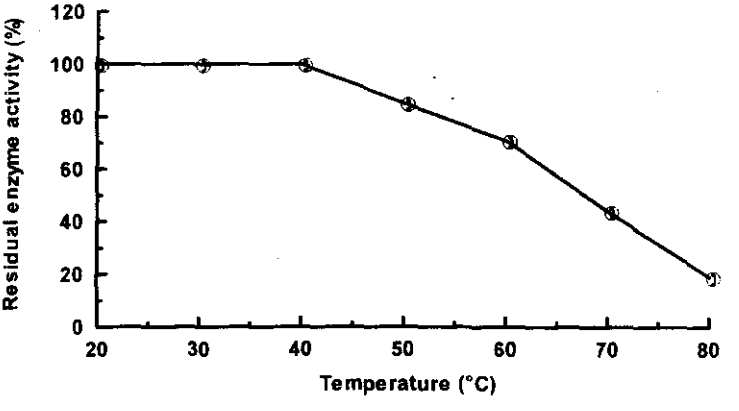

Fig. (7): Thermal inactivation of *B. bifidum* β–galactosidase activity.

pH optima:

Data illustrated in Fig. (8) indicated that the highest enzyme activity occurred in the pH range between 4.5 to 6.5. But, pH 5.0 was found as the optimum pH for enzyme activity. This means that this enzyme was very highly active in the pH range needed for many dairy products ripening. Outside this pH range, enzyme activity declined greatly. Ismail *et al.* (1997) and Shady & Abdel-Razik (1997) found that pH 7.0 is the optimum for enzyme activity.

pH stability:

The enzyme solutions were stored at optimum temperature for one hour. The results recorded in Fig. (9) indicated that the enzyme was completely stable at pH

ranged between 5.0 to 6.0 and lost 5% and 7% only at pH 4.5 and 6.5, respectively. In the alkaline region, enzyme lost highest activity, which lost about 57% of its maximum activity at pH 8.5. These observations showed that, this enzyme may success in the ripening of many dairy products. Dawoud *et al.* (1997b) reported that enzyme was stable at pH 7.0-8.0.

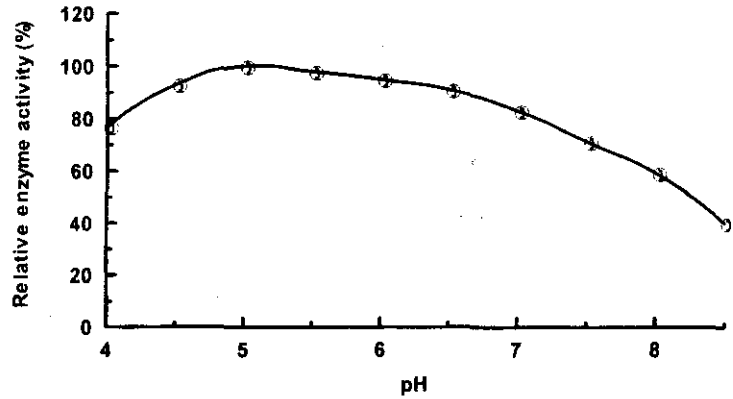

Fig. (8): pH optima for *B. bifidum* β–galactosidase activity.

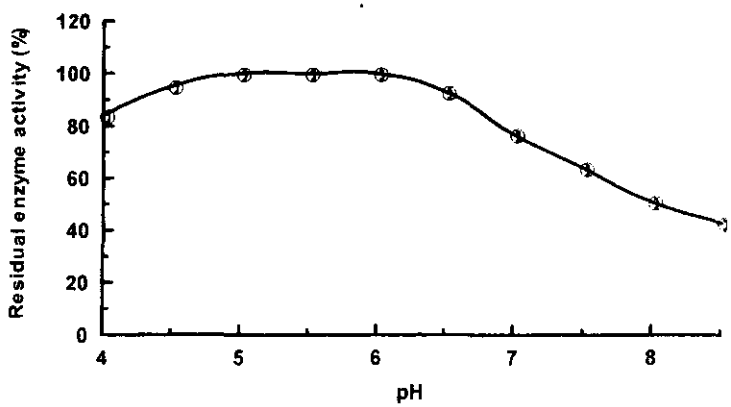

Fig. (9): pH stability of *B. bifidum* β–galactosidase activity.

Effect of some activators or inhibitors:

Some selected metal ions were used at 10^{-2} M concentrations to study the effect of these ions on enzyme activity. The results presented in Table (1) show that the extent of activation or inhibition of β-galactosidase depended on the type of metal ion.

The enzyme was strongly activated by Mg^{+2}, Na^{+}, Ca^{+2} and Mn^{+2}, reaching 33, 27, 18 and 19%, respectively. Therefore, these metal ions appeared to be important for the activation of enzyme protein. On the other side, Fe^{+3}, Zn^{+2}, Cu^{+2} and Fe^{+2} were greatly inhibited the enzyme activity. But, K^{+}, Li^{+} were induced slightly the enzyme activity. Similar results were recorded by Hussain *et al.* (1995) and Ismail *et al.* (1997).

Table 1. Effect of some activators and inhibitors on enzyme activity.

Activators and inhibitors (10^{-2}M)	Relative enzyme activity %	Activators and inhibitors (10^{-2}M)	Relative enzyme activity %
Co^{+2}	105	Zn^{+2}	21
Na^{+}	127	Cu^{+2}	38
Ca^{+2}	118	K^{+}	103
Mg^{+2}	133	Li^{+}	101
Mn^{+2}	115	Fe^{+2}	37
Fe^{+3}	25		

Effect of added enzyme on milk lactose content and titratable acidity:

It is well known that β-galactosidase (lactose splitting enzyme EC 3.2.1.23) hydrolyzes lactose into glucose and galactose producing low lactose dairy products and raising its titratable acidity. Therefore, data presented in Table (2) showed the adding of *B. bifidum* β-galactosidase to fresh pasteurized milk revealed that the increasing of enzyme concentration produced over hydrolysis of lactose content reached 74% with 20 units of enzyme. Highest amount of acidity was also produced. Decreasing in lactose content was also pronounced with the increasing of enzyme concentration. Therefore, the addition of a probiotic bacteria (Bifidobacteria) or its β-galactosidase enzyme to dairy products declined the lactose content, thus making the milk or dairy products more suitable for lactose intolerance and increasing the stability of frozen concentrated whole milk and reduce the sandy texture in some dairy products such as ice-cream. Similar observations were recorded by Ismail *et al.* (1997) and Shady & Abdel-Razik (1997).

Table 2. Effect of adding β-galactosidase on milk lactose content and titratable acidity.

Treatment NO.	Enzyme Conc. (Units)	Lactose content (%)	Hydrolysis rate (%)	Titratable acidity (%)
Control	0.0	5.02	0.0	0.16
I	5	3.60	26.0	0.17
II	10	2.14	57.0	0.19
III	15	1.80	68.0	0.21
IV	20	1.40	74.0	0.24

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بعض خواص إنزيم البيتاجالاكتوسيديز المنتج من البافيدوباكتريم بافيديم وإستخدامه فى تحليل سكر اللبن

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تعتبر بكتيريات البافيدوباكتيريم من أهم الميكروبات المستخدمة فى مجال الصناعات اللبنية لما لها من فوائد جمة سواء صحية أو غذائية حيث قدرتها العجيبة على منع حدوث الإسهال للأطفال وكذلك للبالغين وتحلل سكر اللاكتوز مما يحد من الإضطرابات المعوية كذلك فائدتها العظيمة فى منع حدوث السرطان كنتيجة طبيعية لقدرتها على إفراز إنزيمات البيتاجلاكتوسيديز والنيتروربدكتيز والآزوريدكتيز علاوة على إفرازها للأحماض العضوية والمضادات الحيوية ذات الفائدة الكبيرة فى المحافظة على صحة الفرد ، ولما كان لإنزيم البيتا-جلاكتوسيديز أهمية حيوية لحوالى ٣٠% من الأطفال على مستوى العالم

نتيجة نقص هذا الإنزيم لديهم فقد هدف هذا البحث لإنتاج هذا الإنزيم الهام صحياً وغذائياً من بكتيريا البافيدوباكتريم ذات الفوائد الصحية والعلاجية ودراسة خواصه لإستخدامه فى تحليل سكر اللبن وقد أوضحت الدراسة النتائج التالية .

١ـ وصل أعلى معدل لإنتاج الإنزيم بعد ٤٨ ساعة تحضين .

٢ـ ٣٧م و pH ٤,٥ هما أحسن الظروف البيئية لإنتاج الإنزيم .

٣ـ وجود سكر اللاكتوز فى بيئة النمو بتركيز ٥% كان محثاً لإنتاج الإنزيم .

٤ـ ٣٠م هى المثالية لنشاط الإنزيم .

٥ـ أظهر الإنزيم درجة ثبات ١٠٠% من أعلى نشاط له عند تحضينه على درجة حرارة وصلت ٤٠م ثم فقد ١٥% من نشاطه عند ٥٠م ثم بدأ النشاط يتناقص بدرجة ملحوظة عند تحضينه على أعلى من ٥٠م ولهذا أعتبر هذا الإنزيم أنه من النوع الغير ثابت حرارياً .

٦ـ كانت درجة pH ٥ هى المثالية لنشاط هذا الإنزيم .

٧ـ إحتفظ هذا الإنزيم بكامل نشاطه عند تخزينه فى مدى من درجات الـ pH تراوح من ٥-٦ ثم فقد الإنزيم ٥ ، ٧% من نشاطه عند درجة ٤,٥ ، ٦,٥ ثم تناقص النشاط بدرجة ملحوظة خارج هذا المدى مما يعنى أن هذا الإنزيم ينجح فى العديد من الصناعات اللبنية .

٨ـ كان للعديد من المعادن مثل الماغنسيوم والصوديوم والكالسيوم والمنجنيز تأثير محث لنشاط الإنزيم فى حين كان للبعض الآخر مثل الزنك والحديدوز والحديديك تأثير مثبط لنشاط هذا الإنزيم .

٩ـ نجح هذا الإنزيم بدرجة عالية فى تحليل سكر اللبن مما أدى إلى حدوث نقص كبير فى محتوى اللبن من سكر اللاكتوز كان هذا النقص فى محتوى اللبن من سكر اللاكتوز فى توازى مع زيادة حدوث التحلل كنتيجة لزيادة تركيز الإنزيم وقد أدى زيادة معدل التحلل لحدوث تخمر بدرجة عالية نتج عنه زيادة فى الحموضة الناتجة .

ولذلك أوضحت هذه الدراسة إمكانية إضافة هذا الإنزيم أو البكتيريات الحيوية المفرزة له للمنتجات اللبنية حتى يقضى على ظاهرة عدم تحلل اللاكتوز وظهور القوام الرملى مما يؤدى إلى إنتاج منتجات لبنية ذو خواص حسية وكيماوية وصحية وعلاجية عالية.