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Production and characterization of β-galactosidase from isolated lactic acid bacteria.

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ABSRACT

Twenty strains of lactic acid bacteria (LAB) were isolated from Kareish cheese in Cairo markets. Results of physiological, morphological and biochemical characteristics revealed several genera such as *Lactobacillus* (40%), *Streptococcus* (35%), *Lactococcus* (15%) and *Leuconostoc* (10%). All strains of *Lactobacillus* and *Streptococcus* were grown in whey and tested for the production of β-galactosidase (β-gal) enzyme at 37 and 43 °C. The results indicated that *Streptococcus thermophilus* (4.33 U/ml) and *Lactobacillus bulgaricus* (4.20 U/ml) were the most efficient β-gal activity producers with specific activity of 2.50 and 2.09 U/mg protein, respectively. *Lactobacillus bulgaricus* and *Lactobacillus reuteri* showed higher reducing sugars content in whey medium at 37 and 43°C with hydrolysis of 85.4% and 85.4%, respectively; while *Streptococcus thermophilus* had the highest reducing sugars content in whey permeate medium at 43°C with hydrolysis of 77.3 %. *Streptococcus thermophilus* had the highest wet weight that produced β-gal activity at 37 and 43 °C in whey medium. β-gal was partially purified using ammonium sulfate precipitation from *Lactobacillus bulgaricus* and *Streptococcus thermophilus* at the level of 30-50% and 30-40% saturation recovered 38.73% and 29.72%, respectively. The purification of β-gal by gel filtration using Sephadex G-100 had high specific activity of 6.28 U/mg proteins with 28.89% of β-gal yield extracted from *Lactobacillus bulgaricus* while 4.96 U/mg protein specific activity of β-gal extracted from *Streptococcus thermophilus* with enzyme recovered of 21.06%. The purified β-gal exhibits an optimal activity at pH 7.0 for both *Lactobacillus bulgaricus* and *Streptococcus thermophilus*; while the optimal temperature at 45 and 50°C for *Lactobacillus bulgaricus* and *Streptococcus thermophilus,* respectively. It could be concluded that these bacterial strains isolated from Kareish cheese having β-gal activity may be suitable for various food industrial applications *e.g*., production of lactose free milk and whey or whey permeate-based media utilization to avoid the environmental pollution.

Keywords: Lactic acid bacteria, β-galactosidase, *Streptococcus*, *Lactobacillus*, whey, permeate.

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INTRODUCTION

Lactic acid bacteria (LAB) used as starters for production of dairy products are the main factors of fermentation and protection of fermentative foods. They also have a significant role in texture and flavor of food products [1]. LAB has a long tradition of use in the food industry and their potential uses as a source of enzymes [2]. Thermophilic bacteria have become an objective of interest for the commercial production of β-gal [3]. Among these, special attention has been paid to LAB because of their GRAS (generally regarded as safe) status [4].

β-galactosidase (β-gal, EC 3.2.1.23, trivially lactase) catalyzes the hydrolysis of lactose, the most abundant sugar in milk and its by-products, into its constituent monosaccharide; glucose and galactose, which are more soluble and sweeter than lactose [5]. β-gal enzyme is ubiquitous in nature and can be derived from various sources such as plants, animal organs and microorganisms. In view of the easy production of highly active and stable enzymes, microorganisms are the source of choice [6]. Commercial β-gal is produced from bacteria (such as *Streptococcus thermophilus* and *Lactobacillus lactis*); yeasts (such as *Kluyveromyces lactis* and *Kluyveromyces marxianus*) and moulds (such as *Aspergillus niger*, *Aspergillus candids* and *Aspergillus oryzae*) [7, 8]. Different strains of LAB were assessed for their β-gal productivity of *Lactobacillus acidophilus* ATCC 4356 and *Lactobacillus delbrueckii* spp. *bulgaricus* ATCC11842 which resulted in the highest production potential [6, 9].

LAB requires numerous growth factors such as reconstituted skimmed milk (RSM) and MRS broth in addition to carbohydrate and nitrogen sources in a growth medium [4] to be used for enzyme production. In search for a suitable and inexpensive medium with readily available components, whey appears as an attractive alternative to RSM [10]. Also, sweet whey appears highly attractive mostly due to relatively high lactose content (3-5%) as well as a rich source of proteins, enzymes, vitamins, bioactive compounds and minerals [11, 12]. Moreover, β-gal utilization includes the treatment of waste waters from dairy industries. Namely, disposal of whey and whey permeates causes huge problems in the environment, owing to the low lactose biodegrade ability and the high biological oxygen demand (BOD). Hence, hydrolyzed whey lactose provides a solution for the problem of pollution [13, 14].

Therefore, the present study aimed to evaluate the suitability of sweet whey and whey permeate as a medium for the production of β-gal from several lactic acid bacteria strains isolated from Kareish cheese from Cairo markets such as *Streptococcus thermophilus, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactobacillus casei* and *Lactobacillus bulgaricus.* Moreover, β-gal was extracted, purified and some biochemical characteristics of the potential microorganisms were investigated.

MATERIALS AND METHODS

Materials and samples:

Five samples of Kareish cheese were collected from Cairo markets and immediately transported to the laboratory for analysis. Whey (4.92% lactose) and permeate (5.82% lactose) were obtained from dairy industry unit, animals production research institute, Ministry of Agriculture, Giza, Egypt. O-nitrophenyl β-D-galactopyranoside (ONPG), o-nitrophenol and Sephadex G-100 were purchased from Sigma-Aldrich, Chemical Co., Inc., Germany. Bovine serum albumin (BSA) was purchased from Mallinckrodf Chemical Co., Inc, France. Dye Coomassie brilliant blue G-250 was purchased from Bio-Rad (Richmond, Calif., USA). All other reagents and chemicals were used in analytical grade.

Isolation and identification of lactic acid bacteria (LAB):

Ten grams of cheese were homogenized with 90 ml of sterile solution containing 2% tri-sodium citrate to make the initial dilution (10^1); the suspension was used for making serial dilutions (10^2-10^7). The diluted samples were plated on selective agar media, *Streptococcus* was isolated on M17 agar medium [15], *Lactobacillus* was isolated on MRS medium [16], *Lactococcus* and *Leuconostoc* were isolated on Elliker agar [17]. Microbiological analysis of Kareish cheese was performed directly or after enrichment by incubation at 30

and 45 °C. The selected strains of LAB were kept on MRS agar, M17 agar and Elliker agar slant at 4 °C and streaked every 4 weeks.

LAB were isolated and identified using morphological, phenotypic and biochemical methods. They were examined microscopically for gram staining and catalase activity [18], CO_2 production from glucose, fermentation of sugars (sucrose, mannitol, rhamnose, sorbitol, maltose and cellulose) as well as the growth on 10 °C, 45 °C, 6.5% NaCl and pH 9.6 according to El-Shafei *et al*. [19].

INOCULUM PREPARATION AND GROWTH CONDITIONS

Synthetic media:

Streptococcus and *Lactobacillus* strains were examined in M17 synthetic broth medium and MRS synthetic broth medium, respectively for β-gal production. Media were inoculated with freshly activated 1% inoculum and incubated at 37°C for 24 h according to Vasiljevic and Jelen [20].

β-Galactosidase (β-gal) production using whey and whey permeate-based medium:

Whey and permeate were deproteinized by heating at 85°C for 10 min after adjusting pH to 4.5 using lactic acid. The heat treated whey was cooled to room temperature and filtered through filter paper Whatman No. 1. The pH of whey and permeate media were re-adjusted to 7.0 and sterilized at 121°C for 15 min then inoculated aseptically with 1% of each organism and incubated at 37 and 43°C for 18 h.

Determination of β-galactosidase activity:

β-gal activity was determined as described by Hsu *et al*. [21]. The reaction mixture was composed of 0.5 mL of extracted enzyme and 0.5 mL of 15 mM o-nitrophenyl β-D-galactopyranoside (ONPG) in 30 mM sodium phosphate buffer (pH 6.8). After incubation for 10 min at 37°C, 2.0 ml of 0.1 M sodium carbonate was added to the mixture to stop the reaction. Absorbance was measured at OD_{420} nm with a spectrophotometer (UV 1201-vis spectrophotometer SHIMDZU, Japan). One unit of β-gal was defined as the amount of enzyme that produced one micromol (µM) of o-nitrophenol per min under the assay condition.

Total reducing sugar (TRS) content:

Total reducing sugars concentration of whey and permeate was measured by the 3, 5-dinitrosalicylic acid (DNSA) method described by Miller [22]. The reaction mixture was composed of 0.5 ml of whey or permeate was added to 0.5 ml DNSA reagent (consists of 1 % 3, 5-dinitrosalicylic acid, 0.2 % phenol, 0.05 % sodium sulfite, and 1 % sodium hydroxide) and mixed well. The mixture was heated in water bath at 100 ˚C for 10 min; there after 1 ml of 40% potassium sodium tartrate was added and then cooled to room temperature. Absorbance of the color developed was measured at 575 nm with a spectrophotometer (UV 1201-vis spectrophotometer SHIMDZU, Japan) and the amount of reducing sugars were determined by using a glucose standard curve prepared using concentrations ranged from 0 to 1.03 µg/ml glucose in distilled water.

Wet weight of isolated microorganisms:

The cells were harvested after 18 h of incubation at 37 and 43 °C by centrifuging at 10,000 rpm for 10 min at 4 °C. The supernatant was considered to be containing extracellular enzymes. The cell pellet was crushed and washed twice with a 30 mM sodium phosphate buffer (pH 6.8) and centrifuged at 10,000 rpm for 10 min at 4 °C. The washed pellets formed after the last centrifugation were used for determination of pellet percent wet weight by weighing the pellet.

Measurement of pH changes:

The pH changes of whey and permeate-based media after 18 h of isolated strains grown at 37 and 43 °C were determined using a pH-meter (Hanna Instruments Model 170300, Ingold, Knick, Germany) by direct reading.

Estimation of protein content:

Protein content was determined colorimetrically at OD_{595} nm using Coomassie brilliant blue G-250 dye according to Bradford [23]. The reaction mixture was composed of 10 µL enzyme extract, 490 µl distilled water, and 500 µL of Coomassie brilliant blue G-250 dye was added. The developed color was measured at OD_{595} nm, using (UV 1201-vis spectrophotometer SHIMDZU, Japan). Bovine serum albumin used as a standard protein in range of 0.0-0.6 mg/ml.

β-Galactosidase enzyme extraction:

After 18 h of incubation, the cells were harvested by centrifuging at 10,000 rpm for 10 min at 4°C. The supernatant was considered to be containing extracellular enzymes. The cell pellet was crushed and washed twice with 30 mM sodium phosphate buffer (pH 6.8) and centrifuged at 10,000 rpm for 10 min at 4°C. The washed pellets were resuspended in 5 mL of 0.2 M sodium phosphate buffer (pH 6.8) for intracellular enzyme extraction using Sodium Dodecyl Sulfate (SDS)-Chloroform treatment: permeabilization of cell membrane was carried out by vortexing 10 ml of the cell suspension in the presence of 100 µL chloroform and 50 µL 0.1% SDS solution for 30 min at room temperature [24]. The suspension was centrifuged at 15,000 rpm for 10 min at 4°C and the supernatant was kept at -20°C until β-gal enzyme assay.

PURIFICATION OF B-GALACTOSIDASE

Ammonium sulphate precipitation:

The crude β-gal extracted from *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were precipitated by addition of solid $(NH_4)_2SO_4$ to 90% saturation according to Colowick and Kaplan [25]. The sedimentary protein was collected by centrifugation at 10,000 rpm for 10 min at 4°C. The supernatant was discarded while the precipitate was re-dissolved in a minimum quantity of 0.2 M sodium phosphate buffer pH 6.8, then the resulted fractions were assayed for β-gal activity and protein content. The highly active fraction was dialyzed against a large volume of the same buffer overnight.

Gel filtration chromatography:

The dialyzed fractions were further purified by being applied on Sephadex G-100 column (2.5 × 37 cm) (Phamacia, Uppsala, Sweden), equilibrated with 0.2 M sodium phosphate buffer pH 6.8 and the sample was eluted with the same buffer at a flow rate of 1.0 ml/min. The recovered fractions were assayed for β-gal activity and the protein was detected at OD_{280} nm, then the rich fractions of β-gal activity obtained were pooled and considered as purified β-gal enzyme.

EFFECT OF PH AND TEMPERATURE ON B-GAL ACTIVITY

β-gal optimum pH:

The enzyme activity was measured at different pH values ranging from 3-9 using 0.2 M citrate buffer (pH 3), 0.2 M acetate buffer (pH 4-5), 0.2 M phosphate buffer (pH 6-7), and 0.2 M Tris-HCl buffer (pH 8-9). The β-gal activity was measured after an incubation period of 10 min at each pH.

β-gal optimum temperature:

Tubes containing the reaction mixture and enzyme extract were incubated at different temperatures ranging from 30 to 70°C for 10 min. The enzyme activity was then assayed at each temperature to define the β-gal optimal temperature.

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RESULTS AND DISCUSSION

Isolation of lactic acid bacteria (LAB):

All twenty strains of LAB isolated from Kareish cheese were gram positive, catalase negative, nonspores, rods or cocci. The morphological, physiological and biochemical analysis of LAB isolates indicated that the strains were divided into four genera: *Lactobacillus* 40% (8 isolates), *Streptococcus* 35% (7 isolates), *Lactococcus* 15% (3 isolates) and *Leuconostoc* 10% (2 isolates) as shown in Fig. (1).

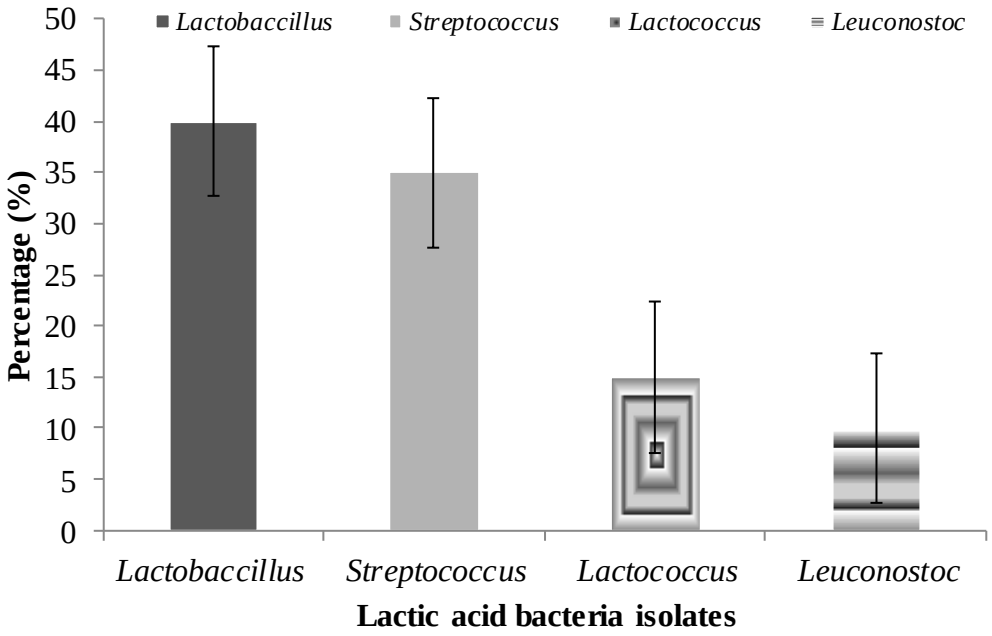

Fig. 1. Percentage (%) of lactic acid bacteria isolated from Kareish cheese.

All *Streptococcus* (7 isolates) were *Streptococcus thermophilus,* while the isolates of *Lactobacillus* were subdivided into *Lactobacillus bulgaricus* (4 isolates), *Lactobacillus casei* (2 isolates), *Lactobacillus reuteri* (1 isolates) and *Lactobacillus rhamnosus* (1 isolates) as shown in Fig. (2).

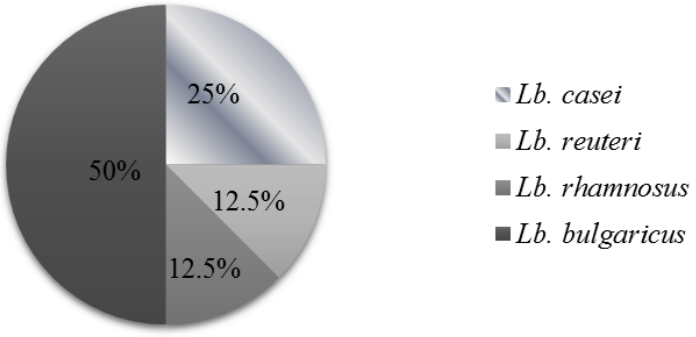

Fig. 2. Distribution of *lactobacillus* strains isolated from Kareish cheese.

El Soda *et al*. [26] reported the presence of a wide variety of LAB in the Egyptian environment. They stated that the isolated and identified LAB show outstanding performances that were similar and in some cases higher when compared to commercially available cultures. In this context, Ibrahim *et al*. [27] detected mesophilic LAB and thermophilic LAB in kareish and Damietta cheese. El Soda *et al*. [26] isolated 28 different

strains of LAB including *Streptococcus thermophilus, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactobacillus casei* and *Lactobacillus bulgaricus* from Egyptian traditional dairy products (raw milk, Kareish, Ras and Damietta cheese, mish, cream, butter, rayeb and zabady). Meanwhile, Hassanein [28] isolated *Lactobacillus casei* subsp. *rhamnosus* and *Lactobacillus paracasei* subsp. *paracasei* from pickled white cheese.

Screening of β-galactosidase (β-gal) activity:

Isolated LAB strains from Kareish cheese were tested for capability of β-gal enzyme production in crude extracts such as, *Streptococcus thermophilus, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactobacillus casei* and *Lactobacillus bulgaricus* grown in whey and permeate-based media examined as a suitable growth medium of β-gal at 37°C and 43°C as shown in Fig. (3). The results showed that *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains were the most efficient β-gal activity producer compared to other strains. Moreover, the incubation temperature at 43°C had the higher β-gal activity than 37°C in all media examined therefore it could be used to produce β-gal enzyme from *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains. Kim and Rajagopal [29] reported that β-gal activity varied up to 2.5 U/ml for *Lactobacillus crispalus,* while β-gal activity extracted from *Streptococcus thermophilus* (4.33 U/ml) and *Lactobacillus bulgaricus* (4.20 U/ml) was higher than previously recorded in whey medium. In addition, Jokar and Karbassi [30] found that the addition of yeast extract (3%), wheat steep liquor (2%) and whey powder (1.5%) into permeate-based medium at 43°C increased the β-gal activity to 4.94 U/ml of *Lactobacillus bulgaricus*. On the other hand, Charitha *et al*. [31] evaluated the optimum temperature for the β-gal production of *Lactobacillus* spp. and found that 35°C enhanced the production of β-gal up to 86 U/ml.

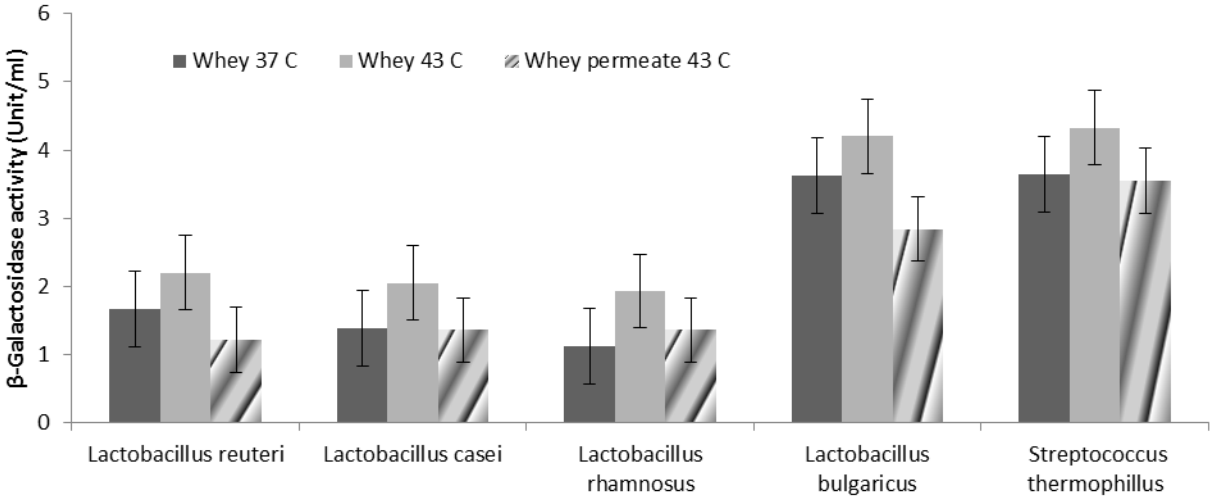

Fig. 3. Effect of whey and permeate-based media on the activity of β-galactosidase extracted from isolated strains at 37 and 43°C.

β-gal production media and growth conditions:

Reducing sugars content and the percentage of lactose hydrolysis (%) in both whey and permeate-based media of all tested strains, *Streptococcus thermophilus, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactobacillus casei* and *Lactobacillus bulgaricus* were grown at 43°C after 18 h as presented in Fig. (4). *Lactobacillus reuteri* (4.20 g/100 ml) showed the highest reducing sugars content followed by *Lactobacillus bulgaricus* (4.06 g/100 ml) in whey medium at 43°C with lactose hydrolysis of 85.4 % and 85.4 %, respectively; while *Streptococcus thermophilus* had the highest reducing sugars content (4.5 g/100 ml) in whey permeate medium at 43°C with lactose hydrolysis of 77.3 %. Moreover, reducing sugars content and the percentage of lactose hydrolysis (%) for all tested strains grown in whey medium in different incubation temperatures 37°C and 43°C after 18 h were showed in Fig. (5). The results showed that *Lactobacillus bulgaricus* (4.20 g/100 ml) had the highest reducing sugars content at 37°C with lactose hydrolysis of 85.4 % compared to other incubation temperature 43°C; also, *Streptococcus thermophilus* had higher reducing sugars (4.5 g/100 ml) with lactose hydrolysis of 77.3 % at 37°C than other incubation temperature at 43°C which resulted 3.9 g/100 ml with lactose hydrolysis of 79.3 %.

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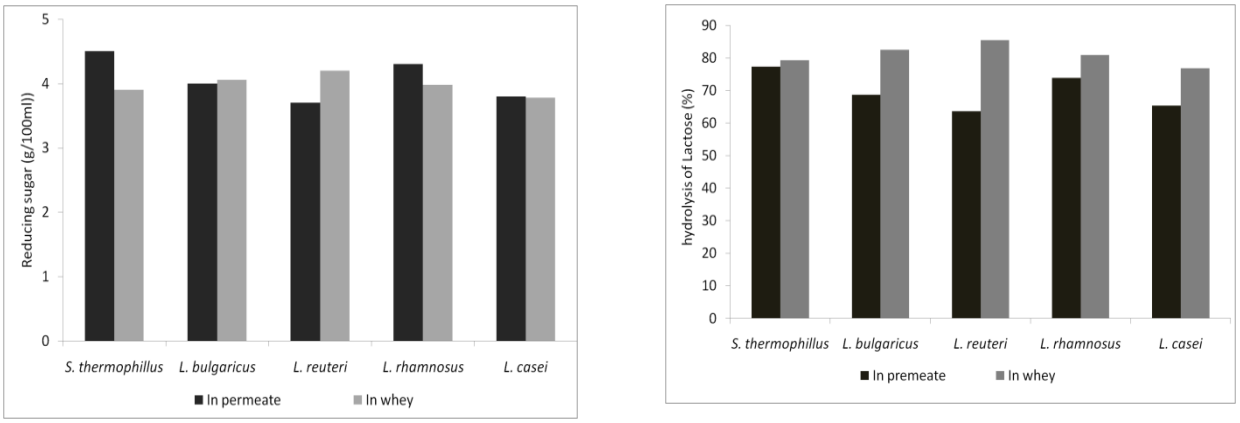

Fig. 4. Determination (a) the reducing sugars (g/100 ml) and (b) the hydrolysis of Lactose (%) in whey and permeate media after 18 h using isolated strains at 43°C.

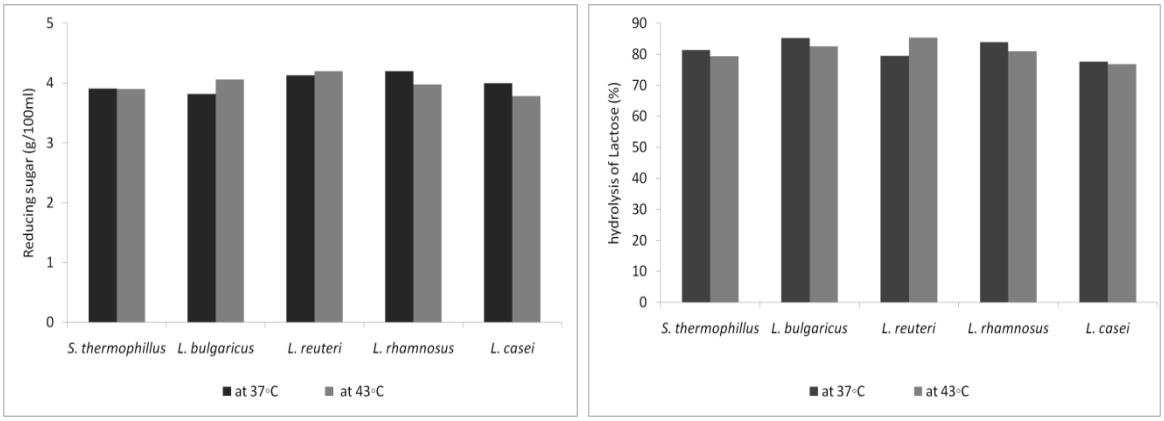

Fig. 5. Determination (a) the reducing sugars (g/100ml) and (b) the hydrolysis of Lactose (%) in whey medium after 18 h using isolated strains at 37◦C and 43°C.

Figure (6) showed that pH changes in whey and permeate-based media after 18 h of isolated strains grown at 37 and 43°C. The results indicated that *Lactobacillus casei* had the lowest pH value of whey growth medium while *Lactobacillus reuteri* had the highest pH value of the same medium at 37 and 43°C. Also, *Lactobacillus casei* had the highest pH value of permeate-based medium while *Lactobacillus reuteri* had the lowest pH value of the same medium at 37 and 43°C.

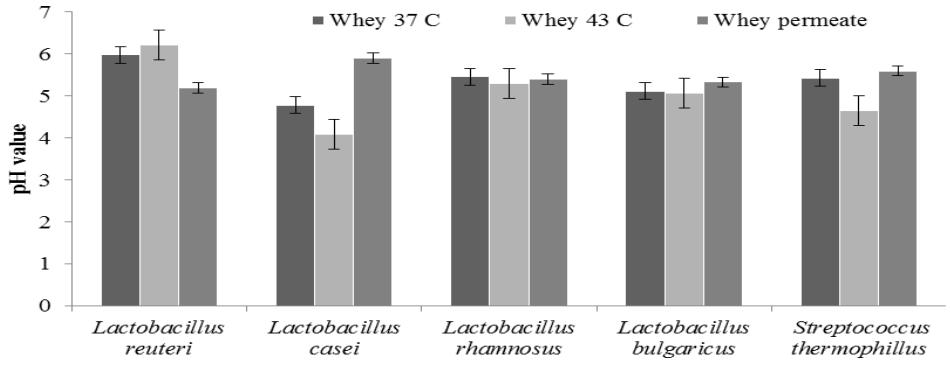

Fig. 6.The changes of pH values in whey and permeate-based media after 18 h of isolated strains grown at 37 and 43°C.

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Wet weight of microorganism's cells that produce β-gal enzyme of all tested strains, *Streptococcus thermophilus, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactobacillus casei* and *Lactobacillus bulgaricus* were grown in whey medium at 43°C after 18 h aspresented in Fig. (7); *Streptococcus thermophilus* had the highest wet weight that produced β-gal activity at 37 and 43°C compared to other microorganisms.

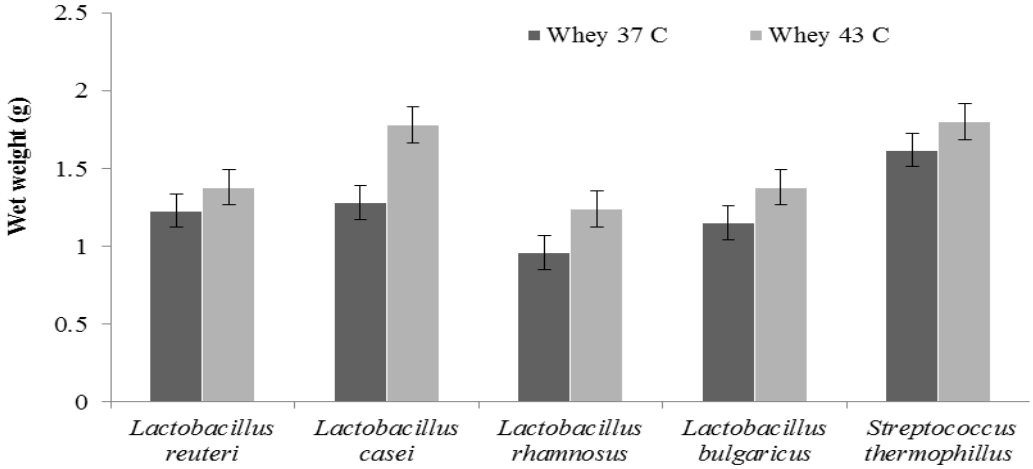

Fig. 7.Wet weight of microorganism that produce β-gal in whey medium after 18 h of isolated strains grown at 37 and 43°C.

Purification of β-gal from selected strains:

Screening of β-gal activity in all tested isolated LAB strains from Kareish cheese showed that *Streptococcus thermophilus* and *Lactobacillus bulgaricus* grown in whey medium at 43°C were the most efficient β-gal producer therefore, specific activity and total activity as critical parameters to select the proper β-gal enzyme producer were determined for all tested strains in whey medium at 43°C as presented in Table 1. The results confirmed that *Streptococcus thermophilus* and *Lactobacillus bulgaricus* had higher β-gal total activity of 64.95 and 63 unit, respectively; moreover, the specific activity was 2.50 and 2.09 unit/mg protein for these strains, respectively than other screened microorganisms. In addition, reducing sugars content and the percentage of lactose hydrolysis of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were higher than other isolated microorganisms in whey medium. Therefore, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains were selected to produce β-gal enzyme which grown in whey medium. In order to produce purified β-gal from the selected strains, β-gal was purified using ammonium sulfate precipitation and gel filtration chromatography on Sephadex G-100.

Table 1. Screening of β-gal activity in crude extracts of isolated strains grown in whey at 43°C.

Microorganism	*Total Activity (unit)*	*Total Protein (mg)*	*Specific Activity (unit/mg Protein)*
Lactobacillus reuteri	***33.00***	***28.35***	***1.16***
Lactobacillus casei	***30.75***	***22.20***	***1.39***
Lactobacillus rhamnosus	***28.95***	***17.55***	***1.65***
Lactobacillus bulgaricus	***63.00***	***30.15***	***2.09***
Streptococcus thermophillus	***64.95***	***25.95***	***2.50***

Specific activity= Enzyme activity/protein content; Total activity= Enzyme activity X fraction volume (15 ml); Total protein= Protein content X fraction volume (15 ml).

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The purification steps, total activity, specific activity and yield of β-gal extracted from *Lactobacillus bulgaricus* are shown in Table (2). β-gal, partially purified using ammonium sulfate precipitation from *Lactobacillus bulgaricus* at the level of 30-50% saturation; recovered 38.73% of the extracted enzyme and improved the specific activity and purification fold to 3.49 U/mg protein and 1.67, respectively compared to other ammonium sulfate fractions. Similar results have been reported by Shaheen *et al*. [32] who purified β-gal from soybean using ammonium sulfate precipitation with 5.1 fold increases in specific activity and 38.3% recovery. The purification of β-gal by gel filtration using Sephadex G-100 (Fig. 8) increased the purification fold with a value of 3.0 compared to previous purification steps and the purified extract had high specific activity of 6.28 U/mg protein with 28.89% enzyme yield.

Table 2. Purification results of β-galactosidase extracted from *Lactobacillus bulgaricus*.

Purification Steps	Volume (ml)	Activity (unit/ml)	Protein content (mg/ml)	Total Activity (unit)	Total Protein (mg)	Specific Activity (unit/mg protein)	Yield (%)	Purification Fold
Crude enzyme	15	4.20	2.01	63.00	30.15	2.09	100	1.00
ASP (30-50%)	10	2.44	0.70	24.40	7.00	3.49	38.73	1.67
GF on Sephadex G-100	100	0.182	0.029	18.2	2.90	6.28	28.89	3.00

Specific activity= Enzyme activity/Protein content; Total activity= Enzyme Activity X Fraction volume; Total protein= Protein content X Fraction volume; Yield= Total activity of purified enzyme/Total activity of crude enzyme X 100; Purification fold= Specific activity of purified enzyme /Specific activity of crude enzyme; ASP, Ammonium sulfate precipitation; GF, Gel filtration chromatography.

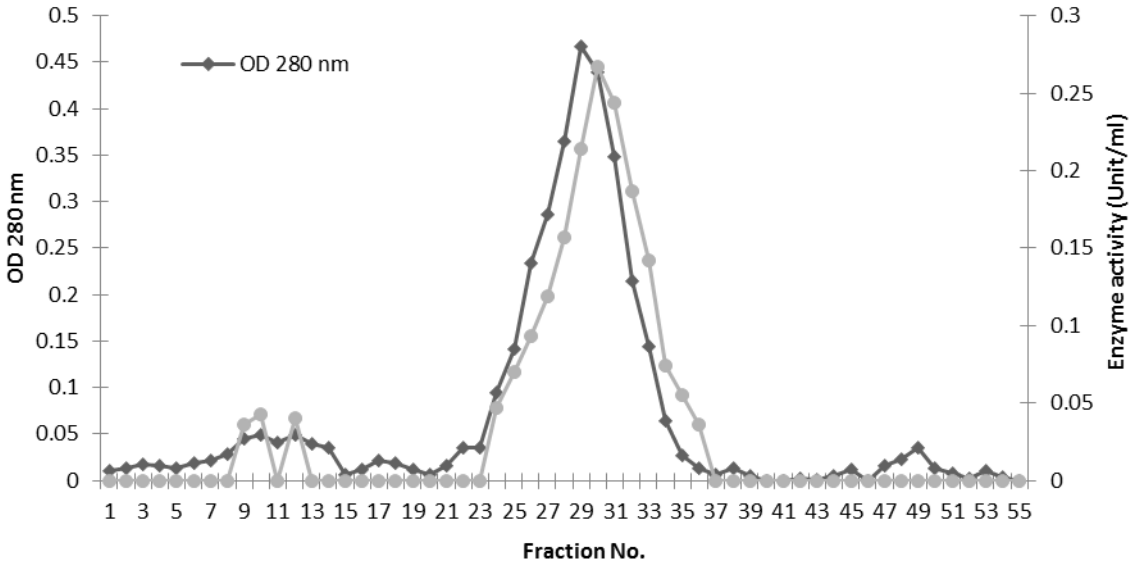

Fig. 8. Gel filtration pattern of *Lactobacillus bulgaricus* β-galactosidase on Sephadex G.100.

Table (3) showed the total activity, specific activity and yield of β-gal extracted from *Streptococcus thermophilus* in all purification steps. The partially purified *Streptococcus thermophilus* β-gal was precipitated with 30-40% of ammonium sulfate saturation and had the highest total activity, specific activity (21.44 U/mg protein), yield (29.72%) and purification fold (8.57). Moreover, the purified β-gal by gel filtration

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chromatography on Sephadex G-100 (Fig. 9) resulted in 1.98 of the purification fold and 21.06 % of β-gal enzyme yield.

Table 3. Purification results of β-galactosidase extracted from *Streptococcus thermophilus*.

Purification Steps	Volume (ml)	Activity (unit/ml)	Protein content (mg/ml)	Total Activity (unit)	Total Protein (mg)	Specific Activity (unit/mg protein)	Yield (%)	Purification Fold
Crude enzyme	15	4.33	1.73	64.95	25.95	2.50	100	1.00
ASP (30-40%)	5	3.86	0.48	19.30	2.40	8.04	29.72	3.21
GF on Sephadex G-100	120	0.132	0.015	15.84	1.80	8.80	24.39	3.52

Specific activity= Enzyme activity/Protein content; Total activity= Enzyme Activity X Fraction volume; Total protein= Protein content X Fraction volume; Yield= Total activity of purified enzyme/Total activity of crude enzyme X 100; Purification fold= Specific activity of purified enzyme /Specific activity of crude enzyme; ASP, Ammonium sulfate precipitation; GF, Gel filtration chromatography.

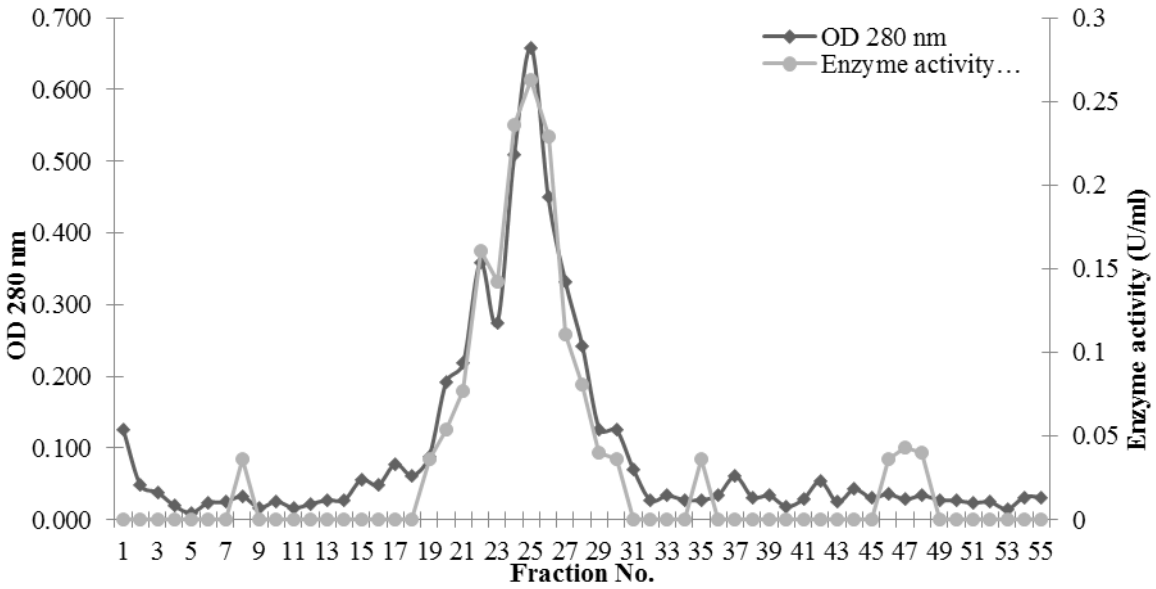

Fig. 9. Gel filtration pattern of *Streptococcus thermophilus* β-galactosidase on Sephadex G.100.

Characteristics of the purified β-gal from selected microorganisms:

Effect of pH and temperature on the purified β-gal activity extracted from *Lactobacillus bulgaricus* and *Streptococcus thermophilus* was studied in order to define the optimum condition of β-gal activity.

Figure (10) showed that the optimum pH of the purified β-gal was 7.0 for both *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* These findings were similar to those of Nagy *et al*. [33], Wang *et al*. [34] and Princely *et al*. [35] who also reported that the highest enzyme activity was observed in the pH range of 6.7 to 7.5. Moreover, optimum β-gal enzyme activity for *Bifidobacterium animalis* and *Lactobacillus bulgaricus* ATCC 11842 [9]; several LAB species including *Lb. acidophilus* [36] was found to be at pH 6.8, but it appeared to have a detrimental effect as the enzyme rapidly lost its activity at a lower and higher scale than this range.

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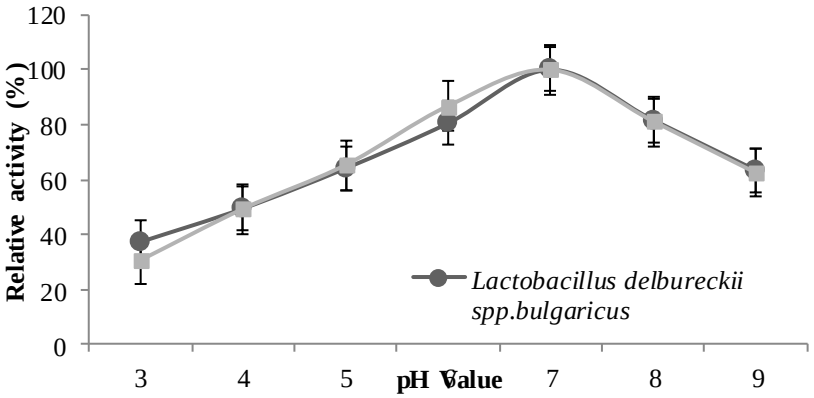

Fig.10.Effect of pH value on β-galactosidase extracted from Lactobacillus bulgaricus and Streptococcus thermophilus.

Effect of temperature changes on β-gal extracted from *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were shown in Fig. (11). The results showed that the optimum temperature of the purified β-gal extracted from *Lactobacillus bulgaricus* was 45°C while 50°C for *Streptococcus thermophilus* β-gal activity. The optimum temperature has been reported in the range of 37 to 45 ºC for maximum β-gal enzyme activity with different organisms [37, 38, 39]. Similar results have been reported for β-gal extracted from *Lactobacillus bulgaricus* ATCC 11842 which showed more enzyme activity at 35°C and 45°C [9]. Also, maximum β-gal enzyme activity from *S. thermophilus* [40], *B. infantis* HL96 [41] and *Penicillium chrysogenum* [33] was obtained at 35-50ºC. Further increase in temperature beyond 50°C resulted in reduction in enzyme activity. Most enzymes denatured rapidly at temperatures above 55°C [42].

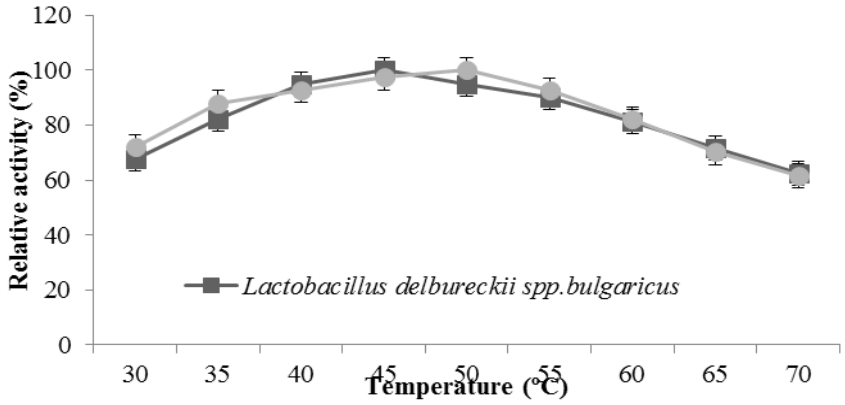

Fig.11. Effect of temperature changes on β-galactosidase extracted from Lactobacillus bulgaricus and Streptococcus thermophilus.

CONCLUSION

It could be concluded that β-galactosidase enzyme was produced from *Lactobacillus bulgaricus* and *Streptococcus thermophilus* strains isolated from Kareish cheese. Moreover, inexpensive and easily available material such as whey or permeate can be effectively used as a substrate for the production of β-galactosidase from yoghurt starter culture that isolated from Kareish cheese which could otherwise be an environmental pollutant, there by contributing to a reduction in the production cost of this enzyme as well as safest environment. Furthermore, the isolated β-galactosidase showed activity over a broad range of temperature and pH, which makes it a promising candidate for food industrial as well as biotechnological applications.

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