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Production of β-Galactosidase Enzyme From *lactobacillus acidophilus* RK Isolated from Different Sources of Milk and Dairy Products

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Abstract : In this paper lactic acid bacteria were isolated from sheep milk, yoghurt, human milk, pasteurized milk and labneh . The isolate bacterial cells were purified , identified and screened to determine their capability for their production of β-galactosidase. *Lacobacillus acidophilus* RK was the best producer.

In a trial to optimize the cultural conditions for maximum â-galactosidase production, different carbon source (glucose was the most suitable carbon source for both intracellular and extracellular enzyme production), acidifying activity (from 0.4 to 0.8). Compare the enzyme production between using free cells and immobilized cells .the immobilized cells were achieved higher production (1.901 U/ml) compared to the enzyme production by free cells (1.192 U/ml) . Studying the physiological and biochemical parameters that increase the production of â-galactosidase by the immobilized organism , 48h. was the best incubation period for both intracellular and extracellular enzymes respectively (1.724 , 1.562 Unit/ml) . The maximum production for the intera crude enzyme obtained from bacterial cells was at pH 4.8 (1.792 U/ml) , the incubation temperature at 40°C was the most suitable temperature for the production of both intra and extra-cellular â-galactosidase production from immobilized cell of *lactobacillus acidophilus RK*. (1.886 and 1.271 U/ml).

Key wards: Isolation, Lactic acid bacteria, 16S rDNA and â-D-galactosidase.

I-Introduction

Lactase (â-D-galactohydrolase) is widely distributed in microorganisms, plants, and animals. It is hydrolyzes the terminal nonreducing â-D-galactoside residues from â-galactosides to a simple monosaccharides glucose and galactose. It has been exploited extensively in the food industries and widely used in the pharmaceutical industry to prevent problems of lactose intolerance by individuals who are lactase deficient.

The lactose- hydrolyzing enzyme, â-galactosidase (â-D-galactosidegalacto hydrolase, trivially lactase) has long been accepted as an important enzyme for dairy industry. It catalyses hydrolysis of lactose, the milk sugar into glucose and galactose and in some cases â-galactosidase is able to catalyze transglycosylation reactions. In dairy industry â-galactosidase has been used to prevent crystallization of lactose, improve sweetness, and increase the solubility of the milk product.

Lactic acid bacteria (LAB) that used as starters for production of dairy products are the main factors of fermentation and protection of fermentative foods and also have a significant role in texture and flavour of food products[1].

As it is well known, lactic acid bacteria (LAB) play a significant role in the food fermentation processes[2]. They are very useful in the food industry owing to their availability to acidify and therefore preserve foods from spoilage.Lactic acid bacteria constitute a various group of microorganisms associated with plants (cabbage, corn, barley, mashes, kale, and silage), meat, and dairy products[3].

Bacterial β-galactosidases are characterized by neutral pH optima as well and they are diverse in their optimum temperature with variation between bacteria and cells even between strains of same bacteria[4,5].

In this study, different species of lactic acid bacteria were isolated and screened to determine their capability for their production and immobilized cells,study the physiological and biochemical parameters that increase the production of β-galactosidase by the immobilized organism.

Materials and Methods

2.1. Sample collection

Samples were taken from the normal habitats of lactic acid bacteria including sheep milk, human breast milk, animal milk, pasteurized milk, yoghurt and also from labanah.

2.2. Isolation and purification of the bacterial isolates

1 gm of all the collective samples diluted and stirred into 100 ml of sterile water for 5min in a 250-ml Erlenmeyer flasks and the suspension was allowed to stand for 30 min. Serial dilutions from each sample were prepared. From the approparaite dilution of each sample, 1 ml was spread on MRS agar medium. The plates were incubated at 30°C for 24 -72 h. The developed colonies were purified and preserved using MRS agar medium[6].

2.3. Screening of all bacterial isolates for β-galactosidase production

All the isolated and purified bacteria were screened for β-galactosidase using MRS agar plates supplemented with 60 µL of 2% X-gal and 2 ml of 2 mM oNPG , 100 µl of appropriate dilutions were inoculated and spreaded over the entire surface of the plates. The plates were incubated in an inverted position for 24 h at 37°C. After incubation, the plates were removed from the incubator and stored at 4°C

2.4. Cultivation of bacteria producing β-galactosidase in liquid media

A 2 stages submerged cultivation was carried out in 250-ml Erlenmeyer flasks contining 50 ml of MRS liquid media (1% peptone, 1% meat Extracts, 0.8% Yeast extracts, 0.4% K2HPO4 ,D (+)glucose 2% , tween 80, 0,01%, sodium acetate,01%, di-ammonium citrate, 0.05, MgSO4.7H2O ,0.2% , MnSO4.4H2O ,0.02% , Initial pH, 6.2) in a shaker (120 rpm) at 37 for 24 h. About 2ml of the preculture ($3x10^8$ CFU/ml) was transferred to 45ml of the same liquid media at pH 6.2. After 48 h samples were taken for determination of bacterial growth at (A_{440} nm) and β-galactosidase activity in both filterate and cells[3].

2.5. Immobilization cell of the selected isolate

A 3% sodium alginate solution was prepared, autoclaved, 3 ml of the bacterial cells were then added to the sterile alginate and mix throughly.The previously prepared alginate cell suspension mixture was dripped into stirred 2% $CaCl_2$ solution (w/v). The calcium alginate beads were cured by stirring $CaCl_2$ for further 2 h. at room temperature, and then transferred to the refrigerator at 5°C overnight in order to increase their stability. The beads were then collected and washed thoroughly using sterile 0.5% NaCl solution (w/v). The beads were transferred to 50 ml of the production (MRS) for further investigation [7].

2.6. Enzyme assay

An assay of β-galactosidases activity was carried out using o-nitrophenylgalactopyranoside (oNPG). One unit of enzyme activity was defined as the enzyme quantity that liberated 1 µmol of o-nitrophenol per minute under the assay conditions.

2.7. Characterization of the selected isolate to genus level

The selected LAB isolate as the best producing organism was characterized and identified. Morphological studies were conducted after growth on MRS agar using light and electron microscopy.

2.8.Phylogenetic analysis using 16S rDNA sequence

Genomic DNA from the selected isolate was obtained using the QIA amp DNA Mini Kit (8). The 16S rDNA gene was amplified by PCR using the forward primer 5'-AGTTTGATCATGGTCAG-3' and the reverse primer 5'-GGTTACCTTGTTACGACT-3'. The DNA sequence was compared to the Gen Bank database at the National Center for Biotechnology Information (NCBI) using the BLAST program.

2.9. Optimization of β-galactosidase production process

The effect of different parametrs on extra and intracellular β-galactosidase production by the selected bacterium KH2 were determined. Optimization studies were selected based on maximum β-galactosidase production. Five different carbon sources (galactose, glucose, fructose, lactose and sucrose) were tested at concentration of 20 g/L for higher β-galactosidase production. Acidification was measured by the change in pH (Δ pH) during incubation time for the selected isolate *Lactobacillus acidophilus* RK cultivated on MRS broth. In a trial to increase the production of β-galactosidase by *Lactobacillus acidophilus* RK , different carbon sources were used , the cell were immobilized by entrapping the cells of *Lactobacillus acidophilus* RK in calcium alginate gel [7].

The effect of incubation period (12, 24, 36, 48, 60 and 72 hours) , different intial pHs (3.6, 4.0, 4.8, 5.4, 6.0, 6.2, 7.2, 7.6, 8, 8.6 and 9) , different temperatures (20, 25, 30, 35, 37, 40 , 45 and 50^{o}C), were determined.

2.10. Statistical analysis

The means of variable and standard deviation were recorded. Data were subjected to statistical analysis using SPSS16, and the difference between mean values as determined by Student,s t-test were $P< 0.05$ considered significant.

3.Results and Discussion

Enzymes serve a wide variety of functions inside living organisms. Lactase also known as β- (abundant disaccharide found in milk) to glucose and galactose, has galactosidase is an enzyme that hydrolyzes lactose a potential importance in the dairy industry . The nutritional value of lactose is limited due to the fact that a large portion such as 50% of world's inhabitants lacks this enzyme and cannot utilize lactose therefore developing lactose maldigestion or intolerance. This however creates a potential market for the application of β-galactosidase. The current share of food enzymes is 863 million dollar in the year 2009, increasing the demand for the discovery of new species, producing enzymes such as β-galactosidase with novel characteristics,which will be of great value to the enzyme industry for different applications. The β-galactosidase enzyme is industrially important because it can be used to avoid lactose crystallization in sweetened, condensed and frozen dairy products. It is also used to avoid the lactose intolerance in individuals who are deficient in lactase [6,9]. On the other hand, researches on lactic acid bacteria has developed greatly in recent years. It is assuming importance in many diverse areas such as biotechnology, nutrition, health, and food safety.

Although β-galactosidase (lactase) has been found in numerous biological systems, microorganisms such as yeasts, mold and bacteria still remain the only sources for commercial. In recent years, thermophilic lactic acid bacteria (LAB) have gained great interest because of their GRAS status (generally regarded as safe) [10]. The β- galactosidase of these cultures has been characterized, showing high stability and activity [2].

The bacterial isolates used from different sources of milk and its derivatives collected from different places. Twenty bacterial isolates were obtained from the normal habitats of lactic acid bacteria on MRS agar medium. All isolates were screened on MRS agar supplemented with 2% X-gal for the production of β-galactosidase. 30% of the screened bacteria were β-galactosidase producer. Some of them produced dark green colonies after 24 h incubation (high enzymatic activity) and others had delay (slow) enzymatic activity after 2 - 4 days of incubation. For all strains, there was a positive correlation between growth and β-galactosidase activity. It was observed that, maximum total of β-galactosidase activity corresponds to the early stationary phase of the six isolates (Figure 1).

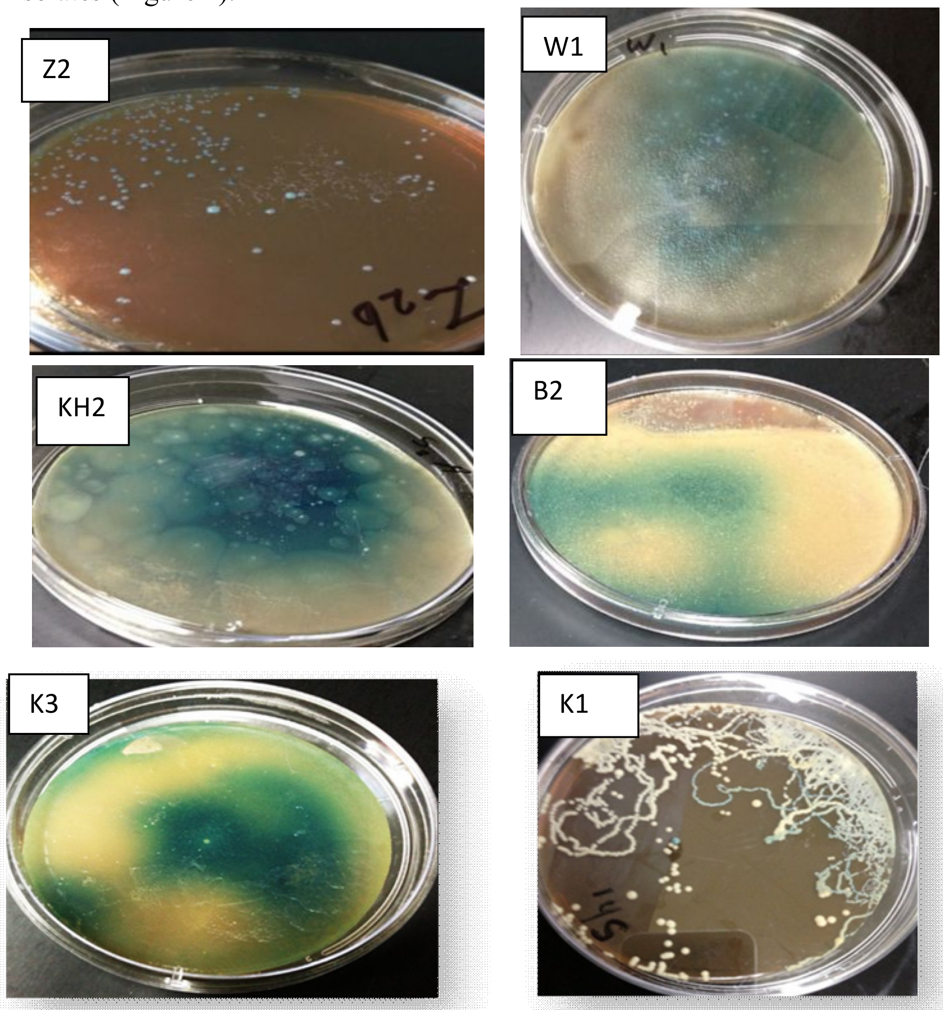

Figure 1- β-galactosidase production byvarious bacteria cultivated on agar plates containing 2% of X-gal

All β-galactosidase producing bacteria were grown in MRS broth for the quantitative determination of β-galactosidase in both supernatant as well as the intracellular fluid. In this study, X-gal and oNPG were used as substrate for detecting β-galactosidase activity qualitatively. Similar technique was used by Kumar [11] and Gheytanchi [1]. On the other hand, Vishwanataha [12] used silica gel thin layer chromatography.

In terms of final cell concentration and as well as production β-galactosidase KH2 isolated from the sheep milk was the most active extracellular producer (Table1and 2). The extracellular enzyme ranged from 0.98 to 1.58U /ml, whereas the intracellular β-galactosidase ranged from 0.61 to 1.33 U /ml. The isolate Z2b had high growth ,however it was weak producer of β-galactosidase.

Table -1: Screening of the isolated bacteria for β-galactosidase production

Organisms	â-galactosidase production		
	24 hours	48 hours	72 hours
Z2	- ve	-ve	-ve
Z2 b	- ve	+ ve	+ ve
Z3	- ve	- ve	- ve
Z4	- ve	- ve	- ve
Z5	++ ve	++ ve	++ ve
B1	- ve	- ve	- ve
B_2	-ve	+ve	+ve
B3	- ve	- ve	- ve
K1	- ve	+ve	++ve
KH2	++ ve	++ ve	+++ ve
K3	++ ve	+++ ve	+++ ve
L1	-ve	-ve	-ve
L2	- ve	- ve	- ve
W4	- ve	-ve	-ve
W2	-ve	-ve	-ve
W1	+++ ve	+++ ve	+++ ve
W3	- ve	- ve	- ve
W5	- ve	- ve	- ve
W6	- ve	- ve	- ve
W7	- ve	- ve	- ve

Table -2: The morphological and microscopically characteristics of LAB, recovered from human breast milk, sheep milk, yoghurt, pasteurized milk

Samples	Isolate symbol	Colony characters		Cell shape	Gram stain
		Shape	Color		
human breast milk	W1	Round, smooth, raised	White	Short rod	+
	W2	Round, smooth, flat	White brilliant	Short rod	+
	W3	Round, smooth, raised	White	Short rod	+
	W4	Round, smooth, flat	Yellowish white- brilliant	Short rod with sheath	+
	W5	Round, smooth, convex	White	Short rod	+
	W6	Round ,smooth, raised	White	Cocci in pairs and chains	+
	W7	Round, smooth, flat	Yellowish white	Short rod	+
Sheep milk	K1	Round, smooth, raised	Yellowish white	Short rod with sheath	+
	KH2	Smooth, round, flat	Grey-white	Large rod	+
	K3	Smooth, round, flat	White	Large rod	+
Yoghurt	Z1	Round, rough, flat	White	Cocci	+
	Z2	Round, rough, raised	White	Cocci in chain	+
	Z3	Round, rough, raised	White	Cocci in chain	+
	Z4	Round, rough, flat	White	Cocci	+
	Z5	Round, rough,	Purple	Larg rod	
Pasteurized milk	B1	Round, smooth, drop-like	White	Cocci in pairs and chains	+
	B2	Round, smooth, flat	White	Cocci in chains	+
	B3	Round, smooth, flat	White	Cocci in chains	+
Labanah	L1	Round, rough, drop-like	Purple	Short rod	+
	L2	Round rough, drop-like	Purple	Cocciin pairs	+

+: Gram positive.-: Gram negative

Examination of KH2 under light microscope and electron microscope revealed that, the isolate was bacilli, nonspore forming bacterium, non-motile, and oxidase and catalase negative. The bacterial cell had a rod shape, It had not either flagella or capsule as shown (Figure 2 a,b). This was in agree with the findings of Muñoz [13]. The diameter of the bacterial cell was 0.7 to 3-5 µm, the colonies were about 1-2 mm in diameter, had milky color and elevated. Similarly, Assefa [14] isolated some LAB from different habitats using MRS agar medium and they were either cocci or bacilli and belonged to Gram-positive bacteria.

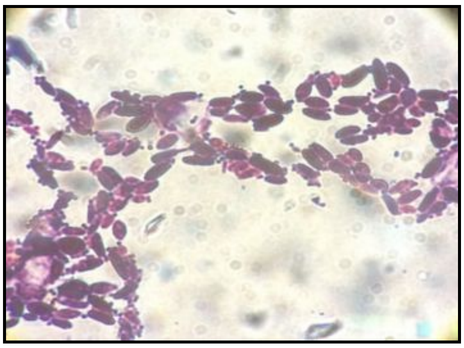

Fig 2a

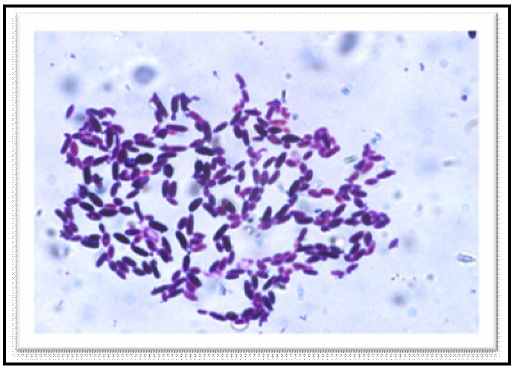

Fig 2b

Fig 2 a,b : Gram stain of the selected isolate KH2 examined light under microscope (x10000).

The selected bacterial isolate was characterized by physiological properties showed in Tables 3and 4 ,showed that the isolates KH2 could grow at temperature range of 20 - 45°C.. It can tolerate NaCl up to 5%. The KH2 organism produced NH3 from arginine and produced acid and gas from sugar. It was homofermentative microbe. Similarly the lactic acid bacteria obtained by Cullimore [15] it grew at temperatures range from 20°C to 45°C. The selected isolate can utilize galactose, glucose, fructose, mannitol, lactose and sucrose. Colinas [16] found similar temperature.

Table 3- .Some physiological and biochemical tests of KH2 isolate

Physiological and biochemical tests	Result
Gram stain	Gram+ve
Spore forming	-
Motility test	-
Oxidase	-
Catalase	-
Hemolysis	-
Starch hydrolysis	+
Lactic acid formation	+
Temperature range	20-45
Minimum temperature	20
Maximum temperature	45
NaCl	0.5-5%
NH_3 from arginine	+
Acid and gas from glucose	+
Glucose fermentation	+
Type of fermentation	Homofermentative

+: Present, - : Absent

Table 4- Biochemical characters of the selected isolate KH2

Tests	Substrate degradation	Results
ONPG	ONPG	+
ADH	Araginine	+
LDC	Lysine	-
ODC	Ornithine	-
CIT	Citrate	-
H_2S	Na thiosulfate	-
URE	Urea	-
TDA	Tryptophan	-
VP	Na pyruvate	-
GEL	Gelatin	+
INO	Inositol	-
AMY	Amygdalin	+
ARA	Arabinose	-
OX	Oxidase	-
IAA	Indole production	-
Amylase production	Amylase	-

+: Present,- : Absent

The biochemical characters of the selected isolate KH2 was shown in Table (5). The selected isolate KH2 was indole and amylase negative. It degraded each of oNPG, araginine, gelatin, amygdalin , urea, tryptophan, Na pyruvate, inositol, arabinose, oxidase., and it also gave –ve results as indole and amylase producer. It can utilize each of galactose, glucose, fructose, mannitol, lactose and sucrose. On the other hand, it could not utilize each of melebiose, raffinose, ribose, rhamnose, sorbitol, xylose, trehalose, and maltose ,

Table 5- Utilization of different carbon sources by the selected isolate KH2

Carbon source	Utilization
Galactose	+
Glucose	+
Fructose	+
Mannitol	+
Lactose	+
Sucrose	+
Melebiose	-
Raffinose	-
Ribose	-
Rhamnose	-
Sorbitol	-
Xylose	-
Trehalose	-

+: Utilized - : Not utilized

According to morphological, physiological and biochemical characteristics the isolate KH2 belongs to the genus *Lacobacillus* . The identification results were confirmed using 16S rDNA which is considered a powerful tool for deducing phylogenetic and evolutionary relationships among bacteria, archaebacteria, and eukaryotic organisms [17]. The DNA sequence was compared to the GenBank database at the National Center for Biotechnology Information (NCBI) using the BLAST program, examined the relevant phylogenetic relationships via the neighbor-joining method. The isolated bacteria was clustered to a type strain, *Lacobacillus*. In addition, the 16S rDNA sequence from strain KH2 evidenced similarities of 97% to that *Lacobacillus acidophilus* XMS111 (Figure 3). In accordance to its biochemical properties and hylogenetic analysis, we concluded that the strain KH2 belongs to *Lacobacillus acidophilus*RK.

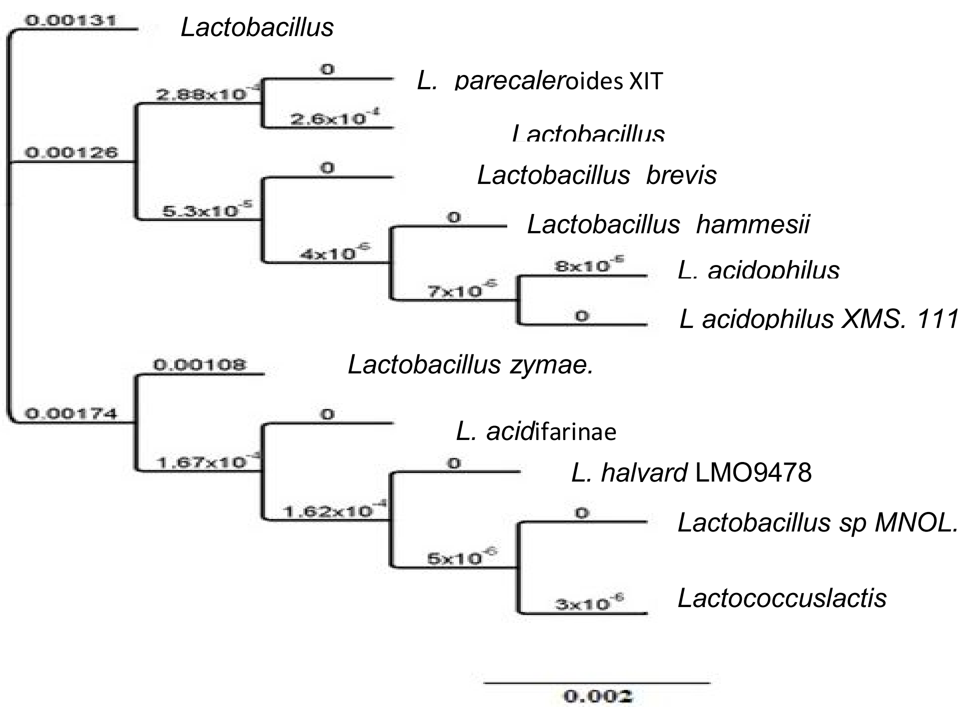

Figure 3- Phylogenetic tree based on 16S rRNA gene sequences

forward primer 5'-AGTTTGATCATGGTCAG-3'
reverse primer 5'-GGTTACCTTGTTACGACT-3'.

Figure 4- The selected bacterial isolate KH2 on MRS medium after24hr

Lactose, the main sugar in milk and whey, and its corresponding hydrolase, β- galactosidase, have been the subject of extensive research during the past decade. The enzyme β-galactosidase has been used to hydrolyze lactose in milk to glucose and galactose [18,19] of which benefit for people who are lactose intolerant. This has led to be many reports studied about the characterization of β-galactosidase enzyme from various microorganisms, in order to improve processes for dairy product from this enzyme.

The carbon source is one of most factor regulating the β-galactosidase activity by catabolite repression or inducer exclusion. Most bacterial cells have the capacity to utilize several carbohydrates as carbon and energy source and posses various transport proteins and catabolic enzymes for the metabolism of the different carbohydrates [20]. The results showed in fig. 5- the maximum enzyme production was attained when glucose was used as a sole carbon source. Many workers found that glucose may enhance or did not have any effect on enzeme activity and that is in agree with our results [21,22,23,24]. But recently, This was on contorary of the findings of each Khleifat [25], who stated that, at fixed concentration (0.5%), lactose and galactose acted as inducers while glucose and other tested carbon sugars showed repression effects on β-galactosidase production in *Enterobacteraerogenes* strain, also Carević [26] used lactose as a sole carbon source for β-galactosidase production using *Lactobacillus acidophilus* ATCC 4356, while Kumar, [11] stated that xylose found to be the better carbon favoring maximum enzyme production. On other studies Rephali and Saier, [27] declared that the glucose present in the external medium exert a repressive effect. Hickey [28] proposed that addition of glucose into growth medium containing lactose decreases the β-galactosidase activity. Also, Akcan [29] found that xylose and galactose supported maximum β-galactosidase production.

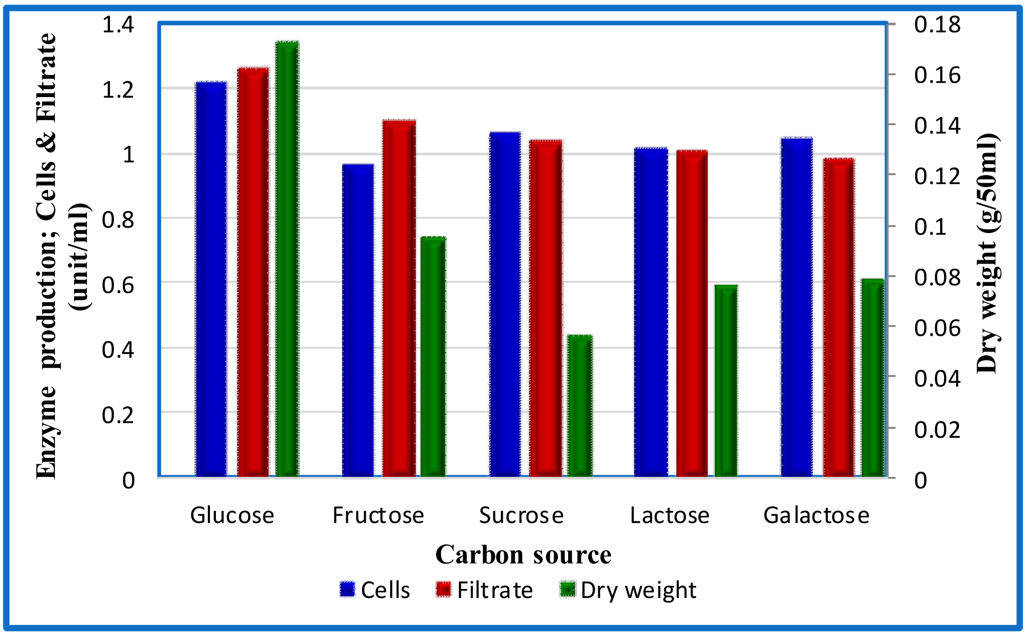

Figure 5-Effect of carbon source on enzyme production byfree cell of *Lacobacillus acidophilus* RK bacterium

In consequent studies, immobilized cells (Fig 6a,b) as well as the optimization of the parameters were studied in a trial to get the best β-galactosidase activity from the isolated strain. Growth and β-galactosidase activity varied by, incubation period, immobilization of cells , initial pH, and temperature.

The production of β-galactosidase enzyme using free cells and immobilized cells entrapped in calcium alginate were used (Figure 6 a ,b) showed the immobilized cells, the results showed in figure 7 indicated that there is a significant difference in the production of extracellular (1.9945Uml) and intercellular (1.192Uml) β-galactosidas enzyme between the immobilized cells which achieved high production compared to the enzyme production by free cells. Accordingly, we continued our study using immobilized cells for the enzyme production.

(Figure 6 a ,b) Immobilized cell with calcium alginate

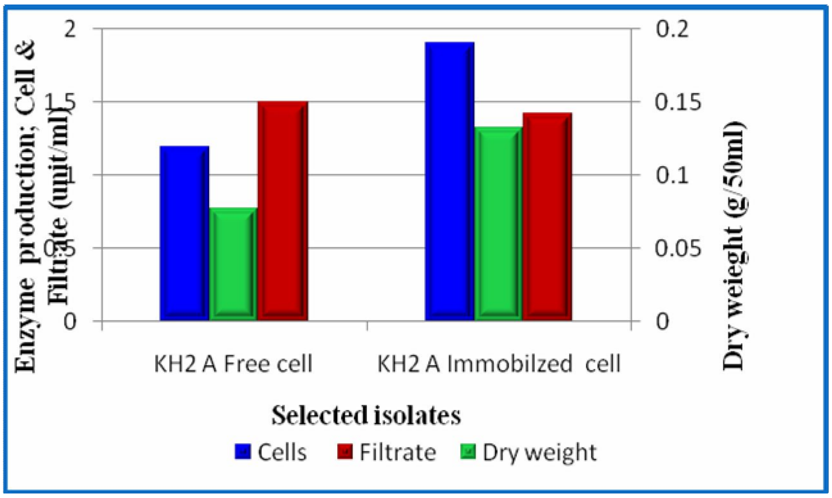

Figure 7- Production of β-galactosidase by immobilized cell of the selected isolate KH2

In similar results Banerjee [30] found that the Immobilized cells retain 68.6% of the β-galactosidase activity of intact cells and there is no significant loss of activity on storage at 4 ^{o}C for 28 days. Russo [31] also declared that the β-alactosidase activity of *Kluyveromyces fragilis* cells immobilized in a k-carrageenan gel was studied in a bioreactor functioning under isothermal and non-isothermal conditions. increased in enzyme activity which proportionaly to the intensity of the temperature gradient applied across the biocatalytic membrane.

The effect of incubation period on β-galactosaidase production by the immobilized selected isolate *lactobacillus acidophilus* RK used to inoculate MRS medium at pH 4.8.The flasks incubated at temperature of 37^{o}C the previous at different incubation periods of 12, 24, 36, 48, 60 and 72 h. Figure (8) illustrated that the β-galactosaidase production increased by increasing the incubation period from 12 up to 48 hours. The β-galactosaidase production by the selected isolate *lactobacillus acidophilus* RK increased until it reached maximum (1.724 , 1.562 U/mL) for both intracellular and extracellular enzymes respectively, after 48 hours of incubation period. There is a parallel relationship existed between enzyme production and the incubation time depending on the phase of growth of the organism. Enzymes is an anabolic metabolite produced in late logarithmic phase of growth or early stationry phase. *Lactobacillus acidophilus* RK gave amiximum production of β-galactosidase enzyme after 48 h. of the incubation period .It gave a higher productivity for both intra and extra enzyme but this period did not enhance the cell biomass.

This results was in harmony with the results of [32,33], while our findings did not agree with those of [35] where they found that the optimal time for β-galactosidase production was 5 h from *Streptococcus thermophilus* cells, isolated from different dairy products.

Table-8-: Effect of different incubation periods on β-galactosidases production by selected isolate

Incubation period (hours)	Enzyme production ± SD		Dry weight g/50ml
	Cell (Unit/ml)	Filtrate (Unit/ml)	
12	0.803 ± 0.06	1.019 ± 0.11	0.031 ± 0.04
24	1.520 ± 0.12	1.324 ± 0.09	0.124 ± 0.05
36	1.601± 0.09	1.371± 0.13	0.131± 0.03
48	1.724 ± 0.14	1.562 ± 0.17	0.213 ± 0.06
60	1.541± 0.12	1.44± 0.12	0.152± 0.04
72	1.387 ± 0.10	1.226 ± 0.13	0.133 ± 0.02

The It is well known that pH influences the velocity of enzyme-catalyzed reaction therefore, the study of the stability of enzymes is an important aspect to consider in biotechnological processes, as this can provide information on the structure of the enzymes and facilitate an economical design of continuous processes in bioreactors. Deactivation mechanisms can be complex, since the enzymes have highly defined structures, and the slightest deviation in their native form can affect their specific activity. Better knowledge of enzyme stability under operating conditions could help optimize the economic profitability of enzymatic processes. The activity and thermal stability of enzymes is influenced by diverse environmental factors[35]. In this point of view, the β-galactosidase enzyme production effect of intial medium pH on the production of β–galactosidase enzyme and cell biomass are shown in Figure 7. The maximum production for the intera crude enzyme obtained from bacterial cells was at pH 4.8 (1.792 Uml) while the optimum extracellular enzyme production and biomass yield was at pH 6.2.

This variation of the optimum pHs between entrapped cells and filtrate may be refered to the protection effect of calcium alginate for the entrapped cells from the surrounding environment. These findings are in accordance with several earlier literature studies. [36,37] showed that the optimum pH for immobilized enzyme was 4.5 from *Bacillus licheniformis* E66. Similarly, Riou [38] identified optimum pH of 5.0. On the other hand Kumar [11] declared that the optimum pH was 7 for high production of β-galactosidase from *Bacillus Sp*. MPTK 121, while Calandri [39] found that pH 10 was to stapilizar dimensional structure of the enzyme in the support by multipoint covalent immobilization.

Table 4.12: Effect of pH on production of β -galactosidase by immobilized *lactobacillus acidophilus* RK cells

pH	Enzyme production		Dry weight g/50ml
	Cell (Unit/ml)	Filtrate (Unit/ml)	
3.6	0.953 ± 0.40	0.862 ± 0.04	0.233 ± 0.12
4.0	1.518 ± 0.19	0.912 ± 0.06	0.399 ± 0.05
4.8	1.792 ± 0.11	0.999 ± 0.06	0.470 ± 0.14
5.4	1.643 ± 0.12	1.090 ± 0.06	0.670 ± 0.04
6.0	1.472 ± 0.07	1.180 ± 0.04	0.720 ± 0.06
6.2	1.370 ± 0.05	1.230 ± 0.12	0.800 ± 0.14
7.2	1.299 ± 0.00	1.059 ± 0.03	0.600 ± 0.06
7.6	1.297 ± 0.03	1.034 ± 0.03	0.510 ± 0.04
8.0	1.253 ± 0.07	1.010 ± 0.03	0.450 ± 0.01
8.2	1.153 ± 0.07	0.961 ± 0.09	0.46 ± 0.09
9.0	1.053 ± 0.07	0.834 ± 0.14	0.330 ± 0.58

For thermal studies effect on production of β-galactosidase ,the immobilized cells of the selected bacterial isolate *lactobacillus acidophilus* RK cells was cultured in 50 ml of MRS broth medium and then, incubated at different temperatures (20,25,30,35,37,40, 45 and 50^{o}C) for 48 hr. Figure 8 indicated that the incubation temperature at 40°C was the most suitable temperature for the production of both intra and extracellular β-galactosidase production from immobilized cells of *lactobacillus acidophilus* RK. The lowest production was observed at temperature of 20°C. While the temperature of 40°C was the best for the growth of the organism, on the contrary temperature of 50°C was the worst temperature for biomass . Similar declared results were in agree with our results. Ramona and Dutta [40] reported optimum temperature of 40°C for the enzyme production from *Streptococcus thermophi*, Makkar [41] reported that optimum temperature of 42°C for β-galactosidase from *Lactobacillus bulgaricus* and Brady [42] found the optimum between 40°C and 50 °C. Kara. [43] showed maximum enzyme activities between 35°C and 40°C, Similar findings of the optimum temperature of the enzyme activities were reported in several literature studies, Jurado [35] found optimum temperature between 35°C to 40°C for β-galactosidase from *Kluyveromyces fragilis*, Rao and Dutta [44] and Alomari [37] found the optimum temperature from *Kluyveromyces lactice* was at 37°C. Also, Shukla [32] found the best temperature was 37°C for the microbial production of β-galactosidase by submerged fermentation using dairy waste from *kluyveromyces marxianus*

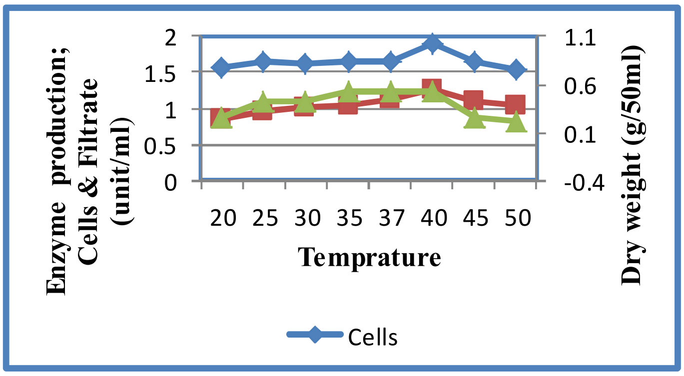

Figure 8: Effects of temperature on growth and production of β- galactosidase by immobilized cell

4-Refrences

1. Gheytanchi, Fariba Heshmati, Bahareh Kordestani Shargh, Jamileh Nowroozi and Farahnaz Movahedzadeh.(2010) Study on β-galactosidase enzyme produced by isolated lactobacilli from milk and cheese Elmira. African Journal of Microbiology Research. 4(6), 454-458.
2. Wood, B.J.B. (1997) Microbiology of fermented foods. 2nd ed. London, Blackie Academic & Professional.
3. Carr, F.J.; Hill, D. and Maida, N. (2002) The lactic acid bacteria: A literature survey. Crit. Rev. Microbiol. 28: 281-370.
4. Gueimonde, M.; Corzo, N.; Vinderola, G.; Reinheimer, J.and De Los Reyes- Gavilan, C.G. (2002) Evolution of carbohydrate fraction in carbonated fermented milks as affected by β-galactosidase activity of starter strains Journal of Dairy Research. 69:125-137.
5. Akolkar, S.K.; Sajgure, A. and Lele, S.S. (2005) Lactase Production from *Lactobacillus acidophilus.World Journal of Microbiology and Biotechnology.* 21:(6-7): 1119-1122.
6. Murugan T, (2013), Solation, Screening and Characterization of β-Galactosidase Enzyme Producing Microorganisms from Four Different Samples.Global Research Journal of Pharmaceutical Sciences, 2 (1) ,12 -14.
7. Fraser, J.F. and Bickerstaff, G.F., (1997) Entrapement in calcium alginate In: Immobilization of enzymes and cells: Methods in biotechnology.(Bickerstaff, G.F., ed.). Humana Press Inc., Totwa, New Jersy, pp. 61 - 66.
8. Weisburg W G, Barns S M, Pelletier D A, Lane D J., (1991) 16S ribosomal DNA amplification for phylogenetic study. 3 (1) ,7 – 16
9. Artolozaga, M. J.; Jones, R.; Schneider, A. L.; Furlan, S. A. and Carvallo-Jones, M. F. (1998) One step partial purification of b-D-galactosidase from Kluyveromyces marxianus CDB002 using streamline-DEAE. Bioseparation. 7:137–143.
10. Holzapfel, W.H.; Haberer, P.; Geisen, R; Björkroth,J; Schillinger,U. (2001) Taxonomy and important features of probiotic microorganisms in food nutrition. Am. J. Clin. Nutr. 73: 365S-373S.

11. Kumar,S., Dhankhar,S., Arya,V. P., Yadav,S., and Yadav* J. P. (2012). Antimicrobial activity of Salvadora oleoides Decne.against some microorganisms. Journal of Medicinal Plants Research. 6(14), 2754-2760.
12. Vishwanataha T., Aishwarya S., Spoorthi N. J.,Divyashree B.C.,Reena V.,Sowmya G., Mohan Kumar B.S.,Venugopa l N.,Sharangowd J .P. And Siddalingeshwara K.G. (2012). A Novel Approach for Screening and Synthesis of β Galactosidase from Microbial Origin.Int. J. App. Biotech and Biochem.,2
13. Muñoz, R; Arena, M. E.; Silva, J. and González, S. N. (2010). Inhibition of mycotoxin producing Aspergillus nomius VSC 23 by lactic acid bacteria and Saccharomyces cerevisiae. Brazilian Journal of Microbiology. 41: 1019-1026.
14. Assefa E.,Beyene F. and Santhanam, A. (2008) Isolation and characterization of inhibitory substance producing lactic acid bacteria from Ergo, Ethiopian traditional fermented milk. Livestock Research for Rural Development.20(3), 1- 6.
15. Cullimore, R. (2000). Practical Atlas for Bacterial identification. Lewis Publishers. London.327 Pages.
16. Colinas, et al., (2014), at Probiotics, Prebiotics, and Synbiotics: Bioactive Foods in Health Promotion, edited by Ronald Ross Watson, Victor R. Preed , published Academic by imprint of Elsevier London, Amesterdam, Oxoford ,Paris.2016.
17. Ölmezoğlu E, Herand B K, Öncel M S, Tunç K, Özkan M., (2012), Copper bioremoval by novel bacterial isolates and their identification by 16S rDNA gene sequence analysis. Turk J Biol. 36: 469 – 476.
18. Yang RZ, andTangs CS (1988). Plants used for pest control in China: a literature review. Econ. Bot. 42: 376-406.
19. Mahoney, R. R. (1998) Galactosyl-oligosaccharide formation during lactose hydrolysis: a review. Food Chemistry. 63: (2):147–154.
20. Poolmen B. (2002) Transporters and their roles in LAB cell physiology Antonie van Leeuwenhock 82: 147-164.
21. Lee, Y. C. and Wacek, V. (1970) Galactosidases from Aspergillus niger. Arch. Biochem. Biophys. 138, 264–271.
22. Park Y K, De Sant M S S and Pastore G M , (1979) , Production and Characterization β-galactodidase from Aspergillus oryzae, J of Food Science,44 (1) 100 – 10
23. Ogushi S, Yoshimoto T, Tsuru D. (1980). Purification and comparison of two types of β-galactosidases from Aspergillus oryzae. J Ferment Technol 58: 115–122.
24. Ohtakara, A., Mitsutomi, M. and Uchida, Y.(1984). Purification and enzymatic properties of alpha-galactosidase from Pycnoporus einnabarinus. Agric. Biol. Chem. 48: 1319-1327.
25. Khleifat K M, Tarawneh K, Wedyan M A and Al-Sharafa K.Y (2006) Growth Kinetics and Toxicity of Enterobacter cloacae Grown on Linear Alkylbenzene Sulfonate as Sole Carbon Source, Current Microbiology 57(4): 364-70.
26. Carević M Vukašinović-Sekulić M , Grbavčić S, Stojanović M, , Mihailović M, Dimitrijević A ,Bezbradica D, (2015) Optimization of β-galactosidase production from lactic acid bacteria. Hem. Ind. 69 (3) 305–312.
27. Rephali, A.W.and Saier, M. H. J. (1980) Regulation of genes coding for enzyme constituents of the Bacterial phosphotransferase system.Journal of Bacteriology. 658-663.
28. Hickey, M. W.; Hillier, A. J.and Jago, G.R. (1986) Transport and metabolism of lactose glucose, and galactose in homofermentative Lactobacilli .Applied and Environmental Microbiology .51 (4): 825-831.
29. Akcan N (2011). High level production of extracellular β- galactosidas from Bacillus licheniforms ATCC 12759 in submerged fermentation. African Journal of Microbiology research. 5 (26), 4615-4621
30. Banerjee ,M., Chakrabarty,A. and Majumdar,S.K. (1982) Immobilization of yeast cells containing β-galactosidase. Biotechnology and Bioengineering. 24 :(8): 1839–1850.
31. Russo, E.B., Medora, R., Parker, K. and Thompson, C., Schedule 1 Research Protocol: An Investigation of Psychedelic Plants and Compounds for Activity in Serotonin Receptor Assays for Headache Treatment and Prophylaxis, Bulletin of the Multidisciplinary Association for Psychedelic Studies, 7 (1997) 48
32. Shukla AK, Manglik A, Kruse A C, Xiao K, Reis R I, Reis W C R I, Staus D P,Hilger D, Uysal S, Huang L Y, Paduch M, Shukla P T, Koide A, Koide S, Weis W I, Kossiakoff A A, Kobilka B K,

Lefkowitz RJ. (2013) Structure of active β-arrestin-1 bound to a G-protein-coupled receptor phosphopeptide. Nature ,497 (7447) 5 – 152

33. Chaturvedi I. , Singh P.K. and Dutta T.K. (2013). Effect of Herbal Feed on Goat Haematological and Biochemical Profile I. J.of Biotech,and Bioeng. Res.4, (3), 257-262
34. Sangwan V, Ali S K T B, Singh R R B, Singh A K. (2015) Production of β-galactosidase from *streptococcus thermophilus* for galactooligosaccharides synthesis ,Journal of Food Science and Technology , 52:(7) 4206–4215
35. Jurado, E.; Camacho, F.; Luzon, G.and Vicaria, J.M. (2004), Kinetic models of activity for β-galactosidase: Influence of pH, ionic concentration and temperature, Enzyme Microbial Technol. 34: 33–40.
36. Onishi N. and Tanaka T ,(1997), Purification and characterization of galacto-oligosaccharide-producing β-galactosidase from Sirobasidium magnum . Letters in Applied Microbiology Volume 24, Issue 2, Version of Record online: 31 Oct. 2003
37. Alomari , W.; Hadadin , M.; Alsaed, A. K. and Al- lsmail, K. (2011) Optmization of Acidic Labnah Whey Lactose Hydrolysis with Immobilized β- galactosidase Enzyme from Kluyveromyces lactis.,Pakistan J. of Nutrition I0 ,(7),675-679.
38. Riou C, Salmon JM, Vallier IZ, Gunata Z & Barre P (1998) Purification, characterization and substrate specificity of a novel highly glucose-tolerant β-glucosidase from Aspergillus oryzae. Appl Microbiol Biotechnol 64: 3607–3614.
39. Calandri C , Marques ,D.P., Mateo C., Carrascosa A.V., Guisán J M , Lorente G F and Pessela B.C. (2013) Purification, Immobilization, Stabilization and Characterization of Commercial Extract with β-galactosidase Activity. J Biocatal. Biotransformation, 2 (1), 1-7.
40. Ramana Rao M.V. and S.M. Dutta.(1977). Production of BetaGalactosidase from Streptococcus thermophilus Grown in Whey. Appl. and Environ. Microbiol. 34: 185–188.
41. Makkar H P S , Sharma O P and Negib S S , (1981), Immobilization and properties of β-D-galactosidase from Lactobacillus bulgaricus . J. Biosci ,3(1) 7 – 16.
42. Brady, D.; Marchant, R.; McHale, L. and McHale, A.P. (1995) Isolation and partial characterization of b-galactosidase activity produced by a thermotolerant strain of Kluyveromyces marxianus during growth on lactose-containing media, Enzyme Microbial Technol. 17: 696–699.
43. Kara, F. (2004) Release and Characterizationof B-Galactosidase from Lactoacillus Plantarum, School of Natural and Applied science of Middle East Technical University .p-122
44. Rao, M. V. and Dutta, S. M. (1977) Production of β-galactosidase from Streptococcus thermophiles grown in whey. *Appl. Environ. Microbiol*. 34:(2) 185.

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