IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 14, Issue 2 Ser. I (Mar – Apr 2019), PP 27-35
www.Iosrjournals.Org

Isolation and identification of *Lactobacillus plantarum* KM5 from camel milk for β-galactosidase production

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Abstract: *β- galactosidase is a magic and promising enzyme, usually produced from microorganisms, plants and animals for different applications like preparation of lactose-free milk for food and dairy products. It was used for hydrolysis of the glucosidic bond of lactose and produced glucose and galactose. Lactose is the main sugar which was found mainly in milk and whey. Bacteria are usually used as a source of the active enzyme. In this research, twenty lactic acid bacterial isolates were obtained from dairy product samples which were collected from Jeddah, Saudi Arabia. All the bacterial isolates were screened for the presence of β-galactosidase on MRS agar medium. Only seven bacterial isolates showed green colons on MRS (Mann, Rogosa and Sharp) agar medium containing X –gal. The amount of β-galactosidase was determined by spectrophotometer using oNPG as substrate. The highest β-galactosidase producer was the isolates KM5 and W1. Comparing the two isolates deeply, isolate KM5 was the best and was selected for more detail studies. A homogenization method was used for the release of β-galactosidase from lactic acid bacterial cells. For characterization of the most active isolate, it was examined under light microscope in addition to determination cell and culture morphology. Identification was determined according to morphological, physiological, and biochemical characters in addition to 16S rRNA.The isolate KM5 isolate was identified as an isolate belonging to genus Lactobacillus and identified as L. plantarum KM5 with 97% similarity level. The effect of different carbon sources on the enzyme production was studied and lactose was the best for growth and enzyme production.*

Key words: *β-galactosidase, Lactic acid bacteria, MRS agar medium, X –gal, Lacobacillus acidophilus*

Date of Submission: 01-03-2019 Date of acceptance:18-03-2019

I. Introduction

β-galactosidase breakdown the substrate lactose, a disaccharide sugar found in milk into two monosaccharide sugars, galactose and glucose. β-galactosidase is an important industrial enzyme in the hydrolysis of milk and whey lactose and the enzymatic hydrolysis of lactose helps in avoiding health and environmental problems posed by this disaccharide. In addition, this enzyme catalyzed the formation of galacto-oligosaccharides, which are prebiotic additives for the so-called "healthy foods". β-galactosidases is one of the relatively few enzymes that have been used in large-scale processes in both free and immobilized forms. Another important outcome of lactose hydrolysis is the increased sweetness and solubility of the resulting monosaccharide, which can generate new applications, especially for whey obtained in large amount as a by-product of the cheese industry (Hensley, 2008) and this enzyme was affected by the presence of metal ions in the media (Banerjee *et al.,* 2018)

As it is well known, lactic acid bacteria are fastidious microorganisms requiring simple carbohydrates as an energy source and amino acids as nitrogen source. *Streptococcus thermophilus* is one of those microorganism has an optimum growth temperature of 40 - 45°C. It ferments limited number of sugars including lactose, fructose, sucrose and glucose (Wessels *et al*., 2004). *Lactobacilli* strains are commonly used in the industry as probiotic. It is well known that β-galactosidase from lactic acid bacteria is an intracellular enzyme, and it is not released to the outside of cells under conventional fermentation conditions (Bury *et al.,* 2001). *Lactobacillus delbrueckii* subsp. *bulgaricus* 11842 can produce relatively high levels of intracellular β-galactosidase in comparison to other dairy cultures (Bury and Jelen, 2000).

Bacterial β-galactosidases are characterized by neutral pH optima as well and they are diverse in their optimum temperature with variation between bacteria and even between strains of same bacteria. Several bacteria have been considered as potential β-galactosidase sources such *as L. lactis, L. acidophilus, L. bulgaricus* (Gueimonde *et al.,* 2002; Akolkar *et al*., 2005). Thermophilic sources have been found to produce

thermo stable β-galactosidase. β-galactosidase from *Streptococcus thermophilus* exhibited twice the activity of enzyme preparations from *Streptococcus fragilis*, being stable at 4°C for more than a year and having high heat stability. Optimum cultural conditions to produce enzyme from *Streptococcus thermophilus* were determined.

Apart from hydrolysis, β-D-galactosidase can catalyze the reverse synthetic reaction of trans-galactosylation, leading to formation of a new glycosidic linkage between two galactose units. Consecutive trans-galactosylation reactions, initiated from galactose and lactose, lead to formation of a galactose unit chain, with terminal glucose unit. These compounds, called galacto-oligosaccharides, are considered probiotics, since they are non-degradable in the upper intestinal tract and pass in the colon, where stimulate the growth and activity of intestinal bacteria (Panesar *et al.*, 2010).

The enzyme β-galactosidase, most commonly known as lactase (Parmjit *et al.*, 2010), is widely distributed in nature in different organisms like animals or plants. Lactase is often confused as an alternative name for β-galactosidase, but it is simply a sub-class of β-galactosidase. β-galactosidase hydrolyses lactose into its monomers that is glucose and galactose, has potential applications in food processing industry. Because of low levels of the enzyme in intestine, large fraction of the population showed lactose intolerance and they have difficulty in consuming milk and dairy products. Lactose has a low relative sweetness and solubility, and excessive lactose in large intestine can lead to tissue dehydration due to osmotic effects, poor calcium absorption due to low acidity, and fermentation of the lactose by microflora resulting in fermentative diarrhea, bloating, flatulence, blanching and cramps, and artery diarrhea, (Husain, 2010).Furthermore, lactose is a hygroscopic sugar, has a strong tendency to absorb flavors and odors, and causes many defects in refrigerated foods such as crystallization in dairy foods, development of sandy or gritty texture, and deposit formation, (Carrara and Rubiolo, 1994).The oxygen bridge connecting the two sides of the lactose molecule is cleaved through the addition of a water molecule. The addition of the water molecule is known as hydrol. The mechanism for how the enzyme actually catalyzes this reaction includes non-covalent interactions, general acid / base catalysis, and electrostatic / covalent stabilization. Microorganisms offer various advantages over other available sources such as easy handling, higher multiplication rate, and high production yield. As a result of commercial interest in *β* galactosidase, a large number of microorganisms have been assessed as potential sources of this Enzyme (Finocchiaro *et al*, 1980; Gul-Guven, 2007; Parmjit *et al.*, 2010).

A large number of bacteria can produce the enzyme β-galactosidase but *Streptococcus thermophilus* and *Bacillus stearothermophilus* are considered as potential bacterial sources. The enzyme from *Escherichia coli* serves as a model for understanding the catalytic mechanism of *β*-galactosidase action, but it is not considered suitable for use in foods due to toxicity problems associated with the host coliform. Hence, the *β*-galactosidase from *E. coli* is generally not preferred for use in food industry (Finocchiaro *et al.*, 1980; Joshi, *et al*., 1989). The metal-activated enzyme, *β*-galactosidase has been isolated from an extremely thermophilic Gram-negative anaerobe and aerobe like *Bifidobacterium bifidum, Clostridium acetobutylicum, Escherichia coli, Klebsiella pneumonia* (Parmjit *et al.*, 2010). Similarly, β-galactosidase was isolated from the non-pathogen, *Arthrobacter oxydans* SB, isolated from India. This study aimed to isolate the β-galactosidase from lactic acid bacteria obtained from milk, whey and dairy milk products.

II. Material And Methods

Collection of samples

Milk, whey and dairy milk samples were collected from supermarkets of Jeddah. The collected samples including human breast milk, sheep milk, cow milk, camel milk, pasteurized milk, yoghurt and white and Labneh cheese.

Isolation of lactic acid bacteria from different sources

A sample of yogurt, Labneh, human breast milk, animal milk, and pasteurized milk, one gram was mixed with 9 ml sterile dist. water (under sterile conditions) and the tubes were shake well to get a dilution of 10:1. Serial dilutions were made, from the appropriate diluents (10^{-3}), 100µl were taken for screening on the selective medium, De Mann, Rogosa and Sharpe (MRS) medium which composed of (g/l) Proteose Peptone 10.0, Beef extract 10.0, Yeast extract 5.0, Dextrose 20.0, Polysorbate 1.0, g Ammonium citrate 2.0, Sodium acetate 5.0, Magnesium sulfate . 0.1, Manganese sulfate 0.05 and Dipotassium phosphate 2.0. For solid medium preparation, 15.0 g/l of Agar were added. All plates were incubated at 37^0C for 2 days and then the obtained organisms were purified (Murugan, 2013).

Purification and preservation of bacterial isolates

The selected bacterial colonies were purified by streaking and sub-culturing on MRS agar plates until pure cultures were obtained , then transferred to slants and preserved at 4^oC . For long preservation (more than six months) strains were kept in MRS broth plus 40% glycerol and stored at -20^oC (Guessas and Kihal, 2004).

Identification of the selected isolate to genus level

The selected isolate in this study was identified to genus level initially by morphological and physiological tests according to Bergey's Manual of systematic of bacteriology (Holt *et al*., 1994).The bacterial isolate KM5 was grown on MRS agar medium. Morphological studies were conducted using light and electron microscopy. Observation was done at 1000 Xto establish the cell shape, presence or absence of spores. Various biochemical tests were performed for the identification of the isolate KM5, these tests include the ability of strain to degrade different materials (Shukla *et al*., 2013), indole test and oxidase tests were also carried.

Acidifying activity

Acidification was measured by the change in pH (Δ pH) during incubation period according **to** Anumukonda and Tadimalla (2010) method. Twenty-five ml of MRS broth was inoculated with 2 ml of an overnight bacterial culture ($3x10^8$ CFU/ml) and incubated at 37^{o}C. The pH was measured after 2 days of growth using a pH-meter. The acidification values were calculated as the difference between pH at the test time and zero time (immediately after inoculation).

Δ pH = pH at test time – pH at zero time

Phylogenetic analysis of 16S rDNA sequence

Genomic DNA of the isolate KM5 was extracted by QIA amp DNA Mini Kit. Amplification of 16S rDNA gene was carried out by PCR in the presence of two primers, the forward primer 5'-AGTTTGATCATGGTCAG-3' and the reverse primer 5'-GGTTACCTTGTTACGACT-3'. The PCR product was purified, and its sequences was determined. The phylogenetic tree was determined based on 16S rRNA gene sequences using the BLAST program at Gen Bank database at the National Center for Biotechnology Information.

Preculture preparation and bacterial growth

MRS culture broth used as preculture medium for the growth of the bacterial isolate in liquid medium. Erlenmeyer flasks (capacity 250 ml) containing 50 ml of the fresh sterile medium was used to prepare the preculture and inoculated with 2 ml of the selected bacterium suspension ($3x10^8$ CFU/ml). The inoculated flasks were then incubated at 37°C and 120 rpm on rotary shaking incubator (PEMED 3525) for 24 hours. At the end of the incubation period, 2ml of this preculture was transferred to another 250 ml Erlenmeyer flasks containing 50 ml MRS broth for production of the enzyme. Bacterial growth was detected by measuring the optical density at 520 nm using UV spectrophotometer (UV- 1650PC spectrophotometric, Shimadzu). All experiments were carried out in triplicate and averages were calculated.

Primary screening on agar plate

MRS agar plates were prepared and 60 µl of 2% X-gal and 2 ml of 2 mM oNPG were put on the center of agar plates and the solution was spread over the entire surface by using sterilized spreader. The plates were incubated at 37^{o}C until all the fluid had disappeared. The bacterial strains were diluted to proper dilution. Then, 100 µl of appropriate dilutions were inoculated and spread over the entire surface of the plates. The plates were incubated in an inverted position for 24 and 48 hrs. at 37^{o}C. After incubation, the plates were removed from the incubator and stored at 4^{o}C for several hours. The blue colonies were appeared if the bacteria had β-galactosidase activity (Sambrook *et al.*, 2001). The isolates which gave positive reaction were selected for details studies (Onladda, 2009).

β-galactosidase quantification in broth medium

From the screening test, seven bacterial isolates showed the maximum production of β-galactosidase were selected to be grown in liquid medium in the 250 ml Erlenmeyer flasks containing 50 ml of the sterile MRS broth mediaand inoculated by 2 ml of the preculture ($3x10^8$ CFU/ml). The flasks were incubated at 37^0 C for 48hr with agitation at 120 rpm. At the end of the incubation period, the growth of inoculated bacterium was measured as described before. Assay of β-galactosidase activity was determined in the cells and filtrates after hydrolysis of *p*-nitrophenyl-β-D-galactopyranoside (PNPG) as described by Van Laere *et al*. (2000).Cells were collected, washed several times and broken dawn. One unit of enzyme is the quantity that liberate one µmol of the substrate per minute under the assay condition. The bacterial isolate, which showed maximum β-galactosidase production, was selected for more detail studies.

Factors affecting β–galactosidase production by the selected isolate

Effect of carbon sources on β–galactosidase production by free cell

De Mann, Rogosa and Sharpe (MRS) broth media without Dextrose (20 g/l) at pH 6.2 and with 20 g/l of different carbon source (galactose, glucose, fructose, lactose and sucrose) were prepared and sterilized in Erlenmeyer flasks (250 ml capacity) each containing 50 ml of the medium. Each flask was inoculated with 2 ml of precultures (3 x 10^8 CFU/ml). The flasks were incubated in a shaker (120 rpm for 48 hrs) at incubation temperature of 37^{o}C. Growth was determined, and cells were harvested as previously mentioned to measure β–galactosidase production in cells and filtrate. Each experiment was run in triplicates and mean value was calculated.

III. Results

Twenty-two samples including fresh sheep milk, camel milk, pasteurized milk, yoghurt, Labneh and human breast milk were collected from different places in Jeddah city, Kingdom of Saudi Arabia. All the previous samples were used to isolate lactic acid bacteria using MRS agar medium. Different bacterial isolates were obtained, purified and maintained on MRS agar at 4^{o}C, to be screened for β-galactosidase production. The results showed that twenty-two bacterial isolates were obtained from the normal habitats of Lactic acid bacteria. Seven bacterial isolates were obtained from human breast milk, two isolates from camel milk whereas 3 isolates were found in sheep milk. Moreover, 5, 3 and 2 isolates were recovered from yoghurt, pasteurized milk, and Labneh, respectively (data not shown).A symbol K (K1, KH2 and K3), were given for bacterial isolates from sheep milk, the symbol KM (KM1, KM5) for isolates from camel milk, Z (Z1, Z2b, Z3, Z4 and Z5) for isolates from yoghurt, the symbol B (B1, B2 and B3) for isolates from pasteurized milk and symbol L (L1 and L2) for Labneh isolates. The symbol W1, W2, W3 and W7 were for bacterial isolates from human breast milk. All the isolates were either Gram positive coccid or bacilli.

Qualitative screening of β-galactosidase-producing bacteria

All the 22 local isolates were screened for their potentiality to produce β-galactosidase enzyme and selecting the efficient enzyme producing strain. This was achieved by culturing the different tested bacterial isolates on MRS agar plates containing 60 µl of 2% X-gal, then 2 ml of 2 mM oNPG were pipetted on to the center of agar plates and the solution was spread over the entire surface by using sterilized spreader. The plates were incubated for 24hrto detect β-galactosidase enzyme. After incubation period, the plates were examined, and reaction was investigated. The results indicated that β-galactosidase production was ranged from very high production (+++) for some isolates, moderate (++) for others, or few (+) and not found (-).Seven isolates of bacteria were found to be best producers of β-galactosidase. The result showed that all of them give blue color colonies on the agar plate containing 60 µl of 2% X-gal after 24 hr. Two isolates from yoghurt, one isolate from pasteurized milk, two isolates from sheep milk, one isolate from camel milk and one isolate from human breast milk. After 48 hours of incubation, most isolates (18 out of 22) showed enzyme production. The most active isolates in β-galactosidase production after 24 and 48 hr were isolatesK1, KM5, K3, Z5,B1,W1 and Z2b that produced the enzyme and give color with the reagent (Table 1).

The seven active isolates were grown in liquid MRS medium, growth and enzyme activity were determined after 48hrs in both cell and culture filtrate. Table 1 and Figure 1 showed that, among the seven examined lactic acid bacterial isolates, both intracellular and extracellular enzyme of the isolate KM5 which was isolated from camel milk, produced quantitatively high β-galactosidase as measured using oNPG as substrate. The minimum intercellular and extracellular enzyme production was achieved by the Z5 and Z2b isolates (Table 1, Figure 1). Similarly, the maximum production of extracellular β-galactosidase was achieved by the isolate KM5. Enzyme production was measured as U/ml and one unit of the enzyme activity was defined as the amount of enzyme releasing one µmol of oNPG per minute under the described conditions.

Table 1. β-galactosidase production by free cell of lactic acid bacteria using oNPG as substrate

Organisms	Source	Enzyme detection on solid medium	Gram reaction	Enzyme production ± SD		Dry weigh (g/100 ml)	Δ pH
				From cell (U/ml)	From filtrate (U/ml)		
Z5	Yoghurt	++	+ve	0.610 ± 0.03*	1.097 ± 0.05*	0.6	- 2.2
Z2b	Yoghurt	++	+ve	0.659 ± 0.05*	0.982 ± 0.06*	0.6	-2.0
KM5 (control)	Camel milk	+++	+ve	1.327 ± 0.08	1.581 ± 0.02	0.8	2.0
K1	Sheep milk	+++	+ve	0.913 ± 0.05*	1.336 ± 0.08*	0.5	-2.3
K3	Sheep milk	++	+ve	0.886 ± 0.03*	1.199 ± 0.15*	0.2	1.88
B1	Pasteurized milk	++	+ve	0.854 ± 0.09*	1.321 ± 0.02*	0.27	1.12
W1	Breast milk	+++	+ve	1.301 ± 0.12	1.562 ± 0.01	0.079	-2.2

very high production (+++), moderate (++) , Gram positive (+ve), *: significant results compared to control

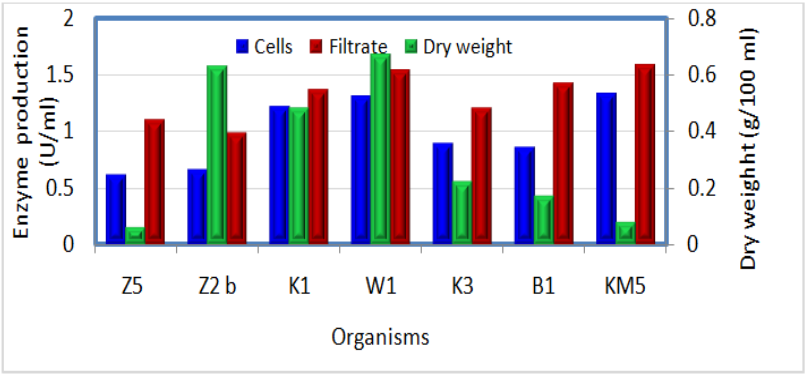

Figure1: Production of β-galactosidase by different free cell of lactic acid

The two highest β-galactosidase producers, the isolates KM5 and W1 were selected and grown in MRS liquid medium for 48hrs in a trial to choose the most efficient isolate in producing of β-galactosidase enzyme. Isolate KM5 produced more β-galactosidase enzymes (1.62 U/ml for filtrate and 1.39 U/ml for cells) compared to the isolate W1 production (1.52 U/ml for filtrate and 1.36 U/ml for cells). The differences between the two isolates in enzyme production was not significant at $p<0.05$. The dry weights were 0.67 and 0.55 g/100 ml for isolate W1 and KM5, respectively. Therefore KM5 was selected as an experimental bacterium for the next studies due to high enzyme production from lower growth (dry weight).

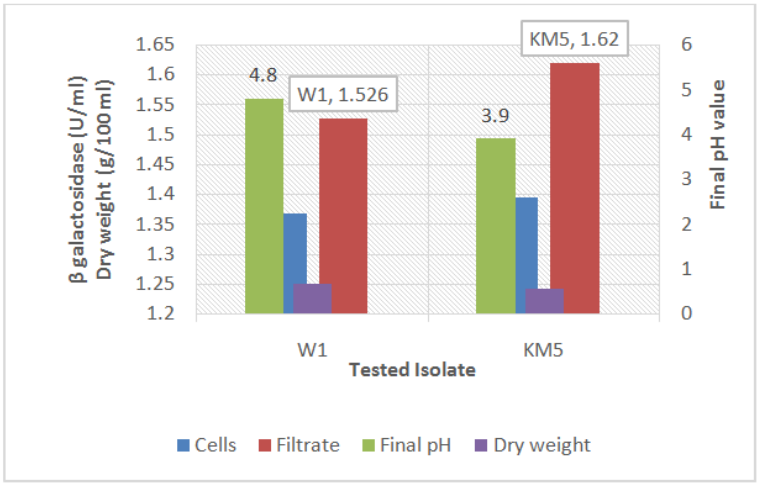

Figure 2. Production of β-galactosidase by cells and filtrates of the most active isolates W1 and KM5

The characteristics of the bacterial isolate KM5which was isolated from camel milk on MRS agar medium were recorded. The selected isolate was Gram positive, non-spore forming bacterium, non-motile and oxidase and catalase negative. It grows well on the previous medium. At first the isolate had small milky creamy color colonies which become yellowish white colored colonies with time. The colonies had rough surface, flat and round. This isolate was identified using morphological and biochemical tests, light and scanning microscopies and automated system for rapid identification of bacteria (Biolog identification system at KAU hospital). The identification was performed according to Bergey's manual of determinative bacteriology. The culture was preserved at 4°C on MRS agar slant and in sterile 40% glycerol at -20°C.

The cells of the selected isolate KM5 were grown on MRS agar, collected stained with Gram stain and examined under light and scanning Microscopes. They were Gram-positive bacilli and positive for β-galactosidase enzyme (Figure 3). Also, it was not spore former bacterium with no flagella or capsule. The diameter of the bacterial cell was 0.6 to 5-10 µm diameters. The isolates KM5produced acid and gas from sugar, had temperature range of 4-45^{o}C and grow well up to 5% NaCl. Some morphological and biochemical characters of isolate KM5 were shown in Table 2. The selected isolate KM5was indole and amylase negative.

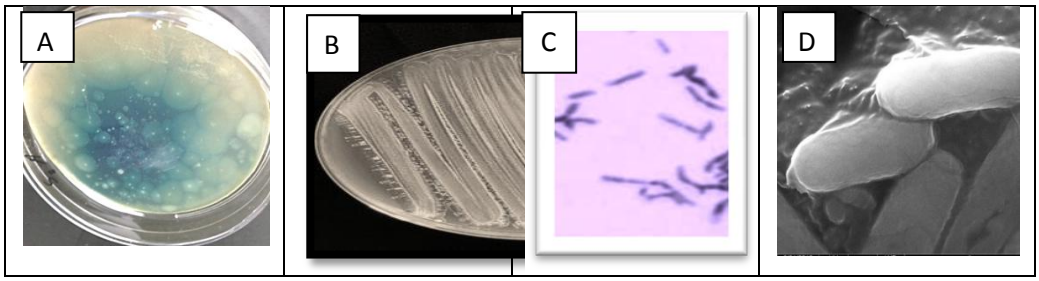

Figure 3: β-galactosidase detection on MRS agar medium after 24 hrs(A), the selected bacterial isolate KM5 on MRS medium (B), udder light microscope X1000 (C) and under electron microscope x 15000 (D).

Table 2. Some morphological and biochemical characters of the selected isolate KM5

Test	Result	Test	Result	Test	Result
Gram	Gram positive	Indole production	-	Amygdalin	+
Shape	Bacilli	Oxidase	-	Arabinose	-
Spore formation	Not found	ONPG	+	Lysine	-
Motility	Not motile	Araginine	+	Ornithine	-

+: Positive result, - : Negative result

The phylogenetic position of the isolate, KM5 was determined after the genomic DNA was extracted using the QIA amp DNA Mini Kit and 16S rDNA gene was amplified by PCR (1451 nucleotides). The sequence was determined and compared to that of gene bank data base which determined the phylogenetic relationships via the neighbor - joining method. Isolate KM5 was belonging to genus *Lacobacillus* with similarty level of 97% to *Lactobacillus plantarum* strain SSK03 and *Lactobacillus plantarum* strain TS1. Lower similarities *Bacillus subtilis* strain S12 and *Bacillus subtilis* ZAA from Gen- Bank database (Figure 3). In accordance to its biochemical properties and phylogenetic analysis, it was concluded that the strain KM5 belonging to genus *Lacobacillus* and identified as *L. plantarum* KM5 {Figure 4).

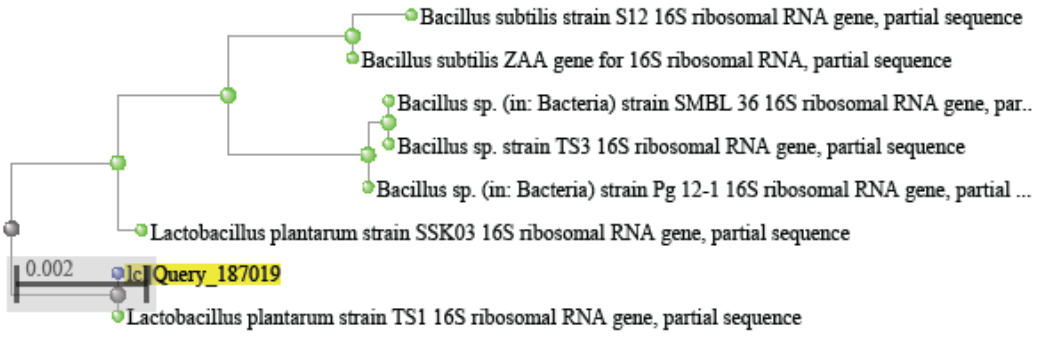

Figure 4: Phylogenetic tree of the isolate KM5 and the most related genera based on 16S rRNA gene sequences

Effect of different carbon source on enzyme production by *Lacobacillus*plantarum KM5

The main research goal of this part of investigation was to determine the best carbon source for β-galactosidase production. This was approached by cultivation the selected bacterial isolate KM5 on modified MRS medium, with different carbon sources (galactose, glucose, fructose, lactose and sucrose) at concentration of 20 g/l. The pH was adjusted to pH 6.8. The flasks were incubated on a rotary shaker at 120 rpm at incubation temperature of 37^{o}C for 2days.

The results were summarized in Figure 4 indicated that isolate KM5 utilize galactose, glucose, fructose, lactose and sucrose. Lactose was the most suitable carbon source for both growth and intracellular and extracellular enzyme production, whereas the other carbon sources showed lower production of intracellular and extracellular β-galactosidase enzyme. There was a slightly variation on the microbial growth indicated that the

microbial growth did not influenced with the kind of the carbon source. So, the microorganism can utilize different kinds of sugars.

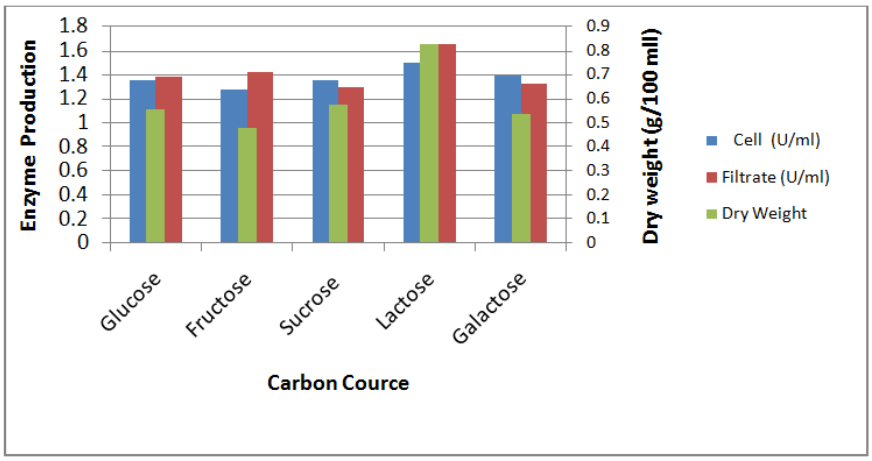

Figure 5. Effect of different carbon source on β-galactosidase production by *Lacobacillus plantarum* KM5

IV. Discussion

β-galactosidase or Lactase is a glycoside hydrolase involved in the hydrolysis of the disaccharide lactose into its constituents of glucose and galactose. This enzyme is essential for hydrolysis milk lactose, thus it is industrially important. It was used to avoid lactose crystallization in sweetened, condensed and frozen dairy products such as ice creams and condensed milk and solve problems associated with whey utilization and disposal. It is also used to avoid the lactose intolerance in individuals who are deficient in lactase (Murugan, 2013). On the other hand, researches on lactic acid bacteria have developed greatly (Hayek et al., 2014). It is assuming importance in many diverse areas such as biotechnology, nutrition, health, and food safety. LAB have been a part of the human diet since ancient times, hence one could argue that they should be completely safe to consume as well as they are bio- preservative of food and feed. The regulations concerning addition of lactic acid bacteria to food vary widely internationally between countries (Wessels *et al*., 2004). Lactic acid bacteria are widely distributed in nature and can be isolated from different habitats. In this connection, different naturally occurring habitats including milk, cheese and Labneh have always been the most powerful source for isolation and obtaining useful culture for enzyme production. This is certainly true for lactic acid bacteria that play an important role in many various traditional industries (Celyk *et al*., 2003).

In the present work, twenty bacterial isolates were isolated from normal habitats of lactic acid bacteria (Twenty samples of fresh sheep milk, pasteurized milk, yoghurt, Labneh and human breast milk) using de-Mann Rogosa Sharpe (MRS) The selected isolate were obtained on MRS medium, a selective culture medium designed to favor the flourishing growth of some lactic acid bacteria and *Lactobacillus* species where sodium acetate prevent the growth of the other bacteria. Out of the twenty-two bacterial isolates, seven bacterial isolates (33%) produced β-galactosidase enzyme on agar medium after 24 hrs incubation (rapid enzymatic activity) which recorded as green color colonies on X-gal plates. Absence of green color indicating no or low β-galactosidase enzyme production under the applied conditions. of incubation .X-gal was introduced as good screening chromogenic β-galactosidases. It is generally used to screen β-galactosidase activity on solid mediums. In this study, oNPG and X-gal were used as substrate for detecting β-galactosidase activity. X-gal was used to test the strains of colonies that produce β-galactosidase; greenish blue color was accepted as positive β-galactosidase activity. The same technique was used by Kumar *et al*., (2012).

Seven isolates showed greenish blue color after 24 hrs so, they considered as a positively producers of β-galactosidase enzyme. The higher qualitatively producers isolates (according to color intensity) were cultured in MRS broth to select the highly potent bacterium for the enzyme production. The highly producer was KM5 and W1 after 24 and 48 hr. It was observed that, maximum β-galactosidase activity corresponds to the early stationary phase of the seven isolates. The most common substrates for assaying β-galactosidase are chromogenic galactosidase. o-nitrophenyl-β-D- galactoside (oNPG) was described as a sensitive and convenient assay, therefore it would be used extensively. Definition of β-galactosidase activity differs considerably.

Throughout this study, a unit activity was defined as the amount of the enzyme required to release one µmol of o-nitrophenol in one minute under the assay conditions.

The highly producers isolates (KM5 and W1) were rescreened to select the higher potentiality isolate. The isolate KM5 was a more efficient than W1 where it gave similar quantities for both intra- and extracellular enzyme, but the cell biomass was lower which mean that fewer cells of the isolate KM5 produce similar quantities of the enzyme compared to isolate W1. Hence the higher β-galactosidase producer isolate KM5 was characterized and identified according to the morphological, physiological and biochemical characters and compared with these described in different identification books and texts. It was a Gram positive, non-spore former, non-motile, and oxidase and catalase negative. This was in agree with the findings of Muñoz *et al*., (2010) and Assefa *et al.*, (2008) who isolated some lactic acid bacteria from different habitats using MRS agar medium and they were either coccid or bacilli and belonged to Gram-positive bacteria. Accordingly, 16S rDNA sequence of isolate KM5 showed 97% similarity level to *Lacobacillus plantarum*. Thus, it was identified as one species of genus *Lacobacillus* and given the name *L. plantarum* KM5. This bacterium can be used for β-galactosidase enzyme production which has been used to hydrolyze lactose in milk to glucose and galactose (Mahoney, 1998, He *et al*., 2005) for people benefit who are lactose intolerant. Characterization of β-galactosidase enzyme from various microorganisms must be done to improve hydrolysis processes for dairy product by this enzyme. Enzymes production of microbial is influenced primarily by the physical parameters of fermentation (temperature, pH, aeration, incubation time). Our results showed the maximum enzyme production was attained when lactose was used as a sole carbon source and lower activities were recorded for glucose. Many workers found that glucose may enhance or did not have any effect on enzyme activity which is not agree with the obtained results but agree with the findings of Khleifat *et al*. (2006), who stated that fixed concentrations of lactose or galactose act as an inducer while glucose and other tested carbon sugars showed suppression effects on β-galactosidase production by *Enterobacter aerogenes*. Kumar, *et al*., (2012) reported xylose as the best carbon source for maximum enzyme production while Hickey *et al*., (1986) found that addition of glucose to growth medium containing lactose, decreased β-galactosidase production. Production of β-galactosidase is primarily carried out using lactose as the substrate. Most reports of optimization of production of this enzyme have used the same substrate (Manera *et al*., 2008; Anumukonda and Tadimalla, 2010; Rashmi and Siddalingamurthy, 2011). In conclusion, researches in the β-galactosidase helped to address the problems faced in the food and allied industries that look for enzymes with novel properties like cold-stability and thermo-active. Novel galactooligosaccharides production by β-galactosidase paved the way for development of prebiotics that can be used as food supplement. Whey utilization by β-galactosidase helped to reduce the water pollution caused by lack of downstream processing and lead to production of products like bioethanol and lactose-hydrolyzed milk. The produced β-galactosidase can be used in many applications and in several industries.

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IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) is UGC approved Journal with Sl. No. 5012, Journal no. 49063.

Khadra Al-Zahrani. "Isolation and identification of *Lactobacillus plantarum* KM5 from camel milk for β-galactosidase production." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 14.2 (2019): 27-35.