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Research Article

# Optimization of Culture Conditions for Production of β-galactosidase from *Lactobacillus acidophilus* Isolated from Dairy Industrial Effluent

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## ABSTRACT

β-galactosidase producing *Lactobacillus* was isolated from dairy industrial waste water collected from Rumuekini, Rivers state. Standard microbiology, biochemical and molecular techniques (16s DNA sequencing) were used for confirmation of the bacterial strain as *Lactobacillus acidophilus*. Macfarlane solution was used for standardization of the microbial inoculum and determination of the heterotrophic counts. P-NPG served as the best substrate for β-galactosidase production from *Lactobacillus acidophilus* with rapid yellow colouration after two days of incubation. Submerged Fermentation (SMF) system was used for the enzyme production. Inoculum size of 2 ml with total heterotrophic counts of $5.4 \times 10^8$ cfu/ml was used throughout the optimization studies. Carbon sources including: lactose, glucose, sugarcane baggase and combination of glucose and lactose were optimized, lactose was found suitable for the protein production with highest β-galactosidase activity (121.71 μmol/min). Among the nitrogen sources optimized, peptone was found optimal for β-galactosidase production with activity of 119.34 μmol/min. pH 6.0 was found the best for the enzyme production. Effect of incubation period on the enzyme production showed the 12th day of fermentation as the peak day for β-galactosidase production from *Lactobacillus acidophilus*. The results from this study have shown that *Lactobacillus acidophilus* isolated from dairy industrial waste water has the potential for β-galactosidase production in a commercial scale for both industrial and clinical applications.

**Keywords:** 16s rDNA; β-galactosidase; Fermentation; Optimization; *Lactobacillus acidophilus*

## INTRODUCTION

β-galactosidase an enzyme of vast multiple applications is a 464-kDa homotetramer protein with 2,2,2-point symmetry. The crystal structure of the enzyme was initially determined in a monoclinic crystal form with the four peptides in the asymmetric unit. Within each monomer, the 1023 amino acids form five well-defined structural domains which include: Domain 1 is a jelly-roll type barrel, Domain 2, Domain 4 are fibronectin type III-like barrels, Domain 5 a β-sandwich and Domain 3 the central is a TIM-type barrel. β-galactosidase can be sourced from: bacteria, fungi, strains of the actinomycetes, plants, animals and human tissues [1]. Among the microbes, lactic acid bacteria *Lactobacilli* are the chief source of the enzyme. In this organism, β-galactosidase is important for production of energy through the breakdown of lactose to galactose and glucose [2]. Large fraction of the world population from epidemiology results are intolerant to milk and dairy products; this is attributed to metabolism and inborn errors in which there is a poor or non-secretion of β-galactosidase within the intestinal epithelial cells. Limitations in utilization of lactose and its further applications in dairy industries are attributed to the following: its ease in crystallization, low relative sweetness and solubility. Clinically, excessive lactose in large intestine can lead to tissue dehydration due to osmotic effects and poor calcium absorption. Fermentation of the lactose by natural microflora results in fermentative diarrhea, bloating, flatulence, blanching and cramps, and watery diarrhea [3]. Furthermore, lactose is hygroscopic and has strong tendency to absorb flavours and odours and causes many defects in refrigerated foods such as crystallization in dairy foods, development of sandy or gritty texture, and deposit formation [4]. Thus the Treatment of milk and other dairy products with β-galactosidase (lactase) is aimed at reducing their lactose content in order to increase their potential uses in the food confectionary industries (in terms of sweetness) and could make milk and other dairy product available to a large

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number of adults and children that are lactose intolerant [5].

Whey is the aqueous fraction of milk generated as bye-products of dairy industries during their bioprocessing [6]. It contains approximately 0.4%-0.5% butterfat, 0.6%-0.8% soluble protein, 4.5%-5% lactose, minerals, trace amount of vitamins and other organic matters [7]. Production of dairy products is a common bio-process and practice around Nigeria. This leads to a considerable amount of dairy whey that poses a serious environmental problem when disposed. Disposing whey in the environment especially in water bodies is problematic because of its high organic content (biodegradable matter) with high Biochemical Oxygen Demand (BOD) and high Chemical Oxygen Demand (COD) [6]. On environmental impact assessments, hydrolysis of whey contents (sugars) by β-galactosidase through galactosylation and transgalactosylation processes can solve therefore mentioned environmental problems associated with whey disposal [8]. Optimization of physiologic parameters for β-galactosidase production is very crucial to meet its economic demand and utilizations [8]. The present study has the objectives of assessing the best physiologic conditions through optimal: bacterial suspension number, incubation period, pH, carbon and nitrogen sources for production of β-galactosidase from isolated *Lactobacilli* isolated from dairy industrial waste water.

# MATERIALS AND METHODS

## Sample collection

Dairy industrial waste water was collected from effluent drainage surge tank of Dairy industry located at Rumuekini front terminal, Portharcourt, Rivers state, Nigeria. The collected waste was taken to the laboratory in a clean sterile sample bottle for further analysis.

## Isolation and identification of strains of *Lactobacilli sp.* from the dairy industry waste water

Strains of the bacteria (*Lactobacilli sp.*) was isolated from the dairy waste water using standard microbiology (culturing/ coliform counting, gram staining and microscopy mounting) and biochemical (sugar fermentations, nitrogen digestions) technique as described by Ezeonu et al. [9].

## Molecular identification of *Lactobacilli sp.*

Genomic DNA (gDNA) from the selected isolate was obtained using the QIA amp DNA Mini Kit. The 16S rDNA gene was amplified by RT-PCR (the conditions for the amplification stated below) using the forward (5'-AGTTTGATCATGGTCAG-3') and reverse (5'-GGTTACCTTGTTACGACT-3') primers. The amplified DNA sequence was compared to the Gen Bank database of National Center for Biotechnology Information (NCBI) using the BLAST program (Table 1).

| Treatment | Tempreture (°C) | Time (minutes) |
|---|---|---|
| Pre-denaturation | 95 | 5 |
| Denaturation | 94 | 1 |
| Annealing | 52 | 1 |
| Elongation | 71 | 7 |
| Final elongation | 72 | 7 |

**Table 1:** Conditions for Amplification of the Bacteria Genome using RT- PCR.

## Screening of *Lactobacilli sp.* for β-galactosidase production

Identified *Lactobacilli sp.* standardized using the Macfarland solution ($BaCl_2/H_2SO_4$) at wavelength of 610 nm and then screened for β-galactosidase producing ability using Demanragoshie sharpie broth supplemented with 2 mM p-NPG as described by Gheytanchi et al. [10]. The inoculated culture broth was incubated at 37°C for 3 days.

## Assay protocol for the enzyme activity

β-galactosidase was assayed according to the method described by Chilaka et al. using p-NPG as the substrate [11]. Assay mixture contained 0.5 ml of enzyme solution, 0.1 ml of 2 mM of p-NPG and 0.5 ml of phosphate buffer solution (pH 6.5). The mixture is incubated at 50°C for 30 min. thereafter, the reaction was stopped using 4 ml of 0.1 M NaOH. Absorbance was taken at 400 nm.

## Protein determination

The total protein content of the enzyme was estimated as described by Lowry et al. using Bovine Serum Albumin (BSA) as the standard protein [12]. Absorbance was taken at 750 nm.

## Optimization of microbial growth rate

Inoculum sizes ranged from 0.5% to 2% v/v used for production of β-galactosidase was incubated for 48 hours at pH 6.0 and room temperature (37°C). Aliquot of the inoculum were collected every 12 hours, viable counts of *Lactobacillus acidophilus* was observed till 48 hours of incubation and standardized using the Macfarland solution.

## Enzyme production

Submerged fermentation technique was used for β-galactosidase production as described by Allam et al. [13]. Production parameters such as: Incubation period, pH, and basic macro nutrients (carbon and nitrogen) were optimized during the production process.

## Optimized incubation period

Fermenters (250 ml) containing 100 ml of liquid media optimized for β-galactosidase production contained: 1% $(NH_4)S0_4$, 0.4% $K_2HPO_4$, 1% Lactose, 0.2 ml tween 80,

0.01% sodium acetate, 01% di-ammonium citrate, 0.05% $MgSO_4.7H_2O$ ,0.2% $MnSO_4.4H_2O$ were incubated at pH 6.0 and at room temperature (37°C) for eighteen (18) days. The whole setups were sterilized at 121°C/15 psi for 20 minutes using the electronic autoclave. Samples were drawn at every 24 hours till 18 days, each drawn sample was filtrated using the muslin cloth of pore size 2 mM and the filtrate (crude extracts) was used to assay for β-galactosidase activity.

## Effect of physiologic pH

As described above, each fermenter containing 100 ml of the liquid production media were incubated at pH of 4.0-8.5 in the range of 0.5 units. Initial pH of the liquid broth was adjusted using 2% HCl v/v and NaOH w/v. They were incubated till the optimal day of β-galactosidase production. The effect of incubation period was determined by incubating production medium at different time intervals (18 days) at optimal production conditions.

## Effect of carbon substrate

Four different rich source of carbon substrates used include: Lactose, glucose, sugarcane baggase and combination of glucose and lactose for the production of β-galactosidase in the liquid medium at 2% w/v concentrations. Fermentation was carried out to the optimal day of enzyme production at the best physiologic condition.

## Effect of nitrogen sources

Four (4) different nitrogen sources used include: Ammonium sulphate, beef extract, peptone and combination of ammonium sulphate and beef extracts for the production of β-galactosidase in the liquid medium at 1% w/v concentrations. Fermentation was carried out to the optimal day of enzyme production at the best physiologic condition.

# RESULTS

The results for the production of β-galactosidase from *Lactobacillus acidophilus* isolated from dairy industrial effluent is shown below Figures 1-7 and Tables 1-3.

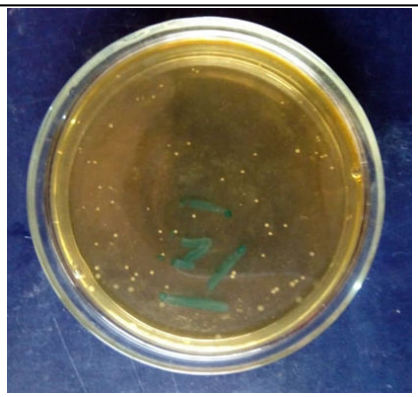

**Figure 1A:** Pure colonies of strains of *Lactobacillus sp.* on MRS medium (2.8 × $10^5$Cfu/m)

| Morphology | Biochemical test |
|---|---|
| Rod shape | Gram positive |
| Smooth and raised colony | Starch hydrolysis (+) |
| Whitish colour colony | Heamolysis (-) |
| Non sporulating | Catalase (-) |
| Non- motile | Lactic acid formation (+) |

**Table 2:** Morphology and Biochemical Characterization of the Bacteria Isolate (*Lactobacilli* sp.)

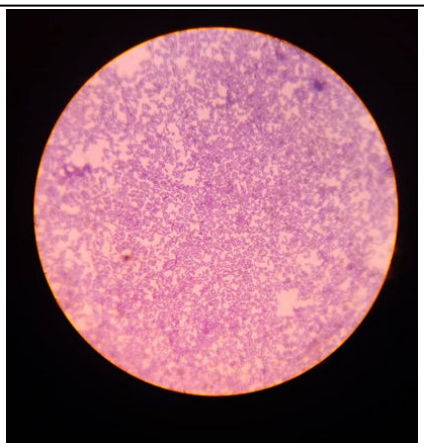

**Figure 1B:** Micrograph of mount strains of *Lactobacilli sp.* under the objectives of light microscope of ×100 magnification.

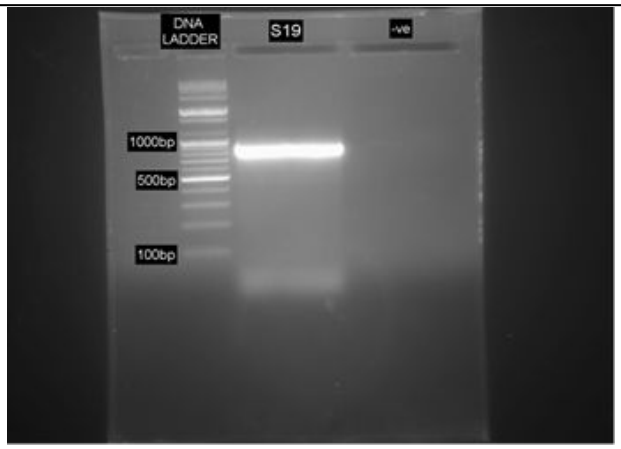


**Figure 2:** Electrophoretogram of the amplified genomic DNA viewed on a UV trans-illuminator.

| Sample | NCBI Identification Specie Name | Query cover | Total score | E value | similarity | Ascension number |
|---|---|---|---|---|---|---|
| Lac.M | *Lactobacilli* | 97 | 2728 | 0 | 99.54 | JX255677 |

**Table 3:** Molecular properties of the identified strain of *Lactobacilli sp.*

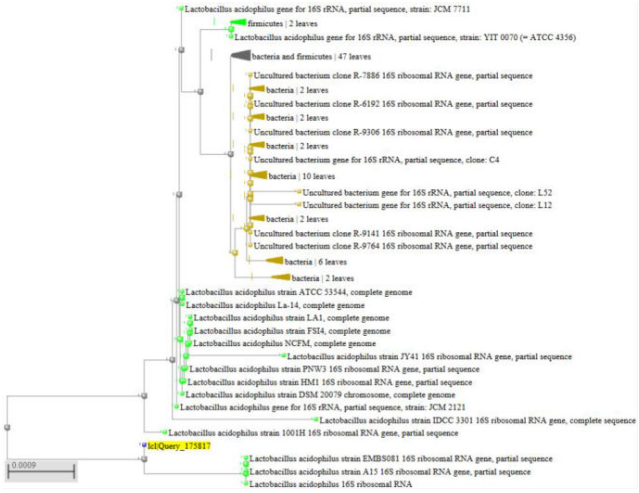


**Figure 3:** Phylogenic evolutionary relatedness of strains of *Lactobacilli* sp. obtained using NCBI BLAST tools.

## Optimization of production parameters for enzyme production

The below graph in the figure below describes the optimization of production parameters for enzyme production.

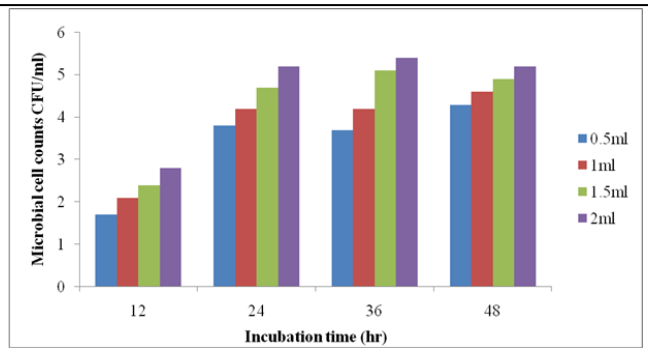


**Figure 4:** Effect of incubation days on the microbial cell Counts (CFU/ml) at various inoculum sizes.

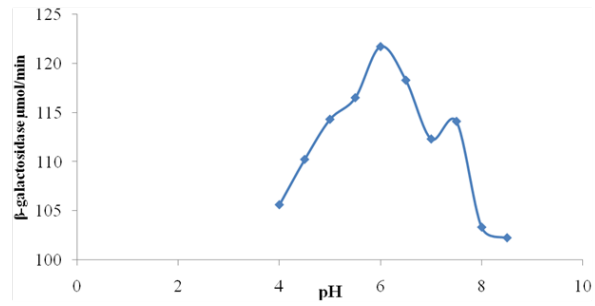


**Figure 5:** Effect of incubation period on β- galactosidase production from *Lactobacilli* acidophilus in a submerged fermentation system

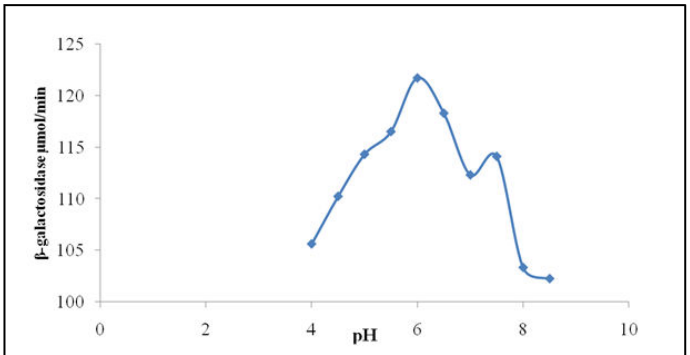


**Figure 6:** Effect of physiologic pH on production of β–galactosidase from *Lactobacilli* sp. incubated for twelve days (12 days) in a submerge fermentation system using standard lactose as the sole carbon source.

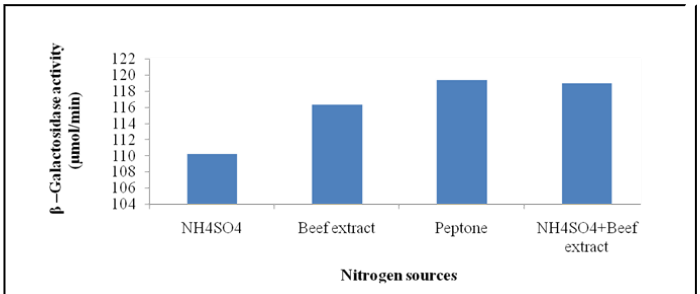


**Figure 7:** Effect of Nitrogen (nutrient) sources on production of β –Galactosidase from *Lactobacilli* sp. incubated for twelve days (12 days) at pH 6.0 in a submerge fermentation system using standard Lactose as the sole carbon source.

# DISCUSSION

Effluent surge tank of the dairy industry located at Rumuekini, Rivers state showed high heterotrophic microbial diversity and activity. Among the microbes isolated, bacteria kingdom was optimal in diversity. Strains of *Lactobacilli sp.* showed greater coliform presence among all other microbes on both nutrient and MRS media with microbial population of $2.8 \times 10^8$ CFU/ml and $1.65 \times 10^7$ CFU/ml respectively. Basic morphological and Biochemical screening were used to identify the isolate as a *Lactobacilli* genre organism. Molecular tests (16s rDNA.) was used to identify the pure isolates of *Lactobacilli*. Electrophoretogram of the amplified genome of *Lactobacilli* using RT-PCR showed a typical base-pair of a bacterial with 750 bp from the ladder DNA segment Figure 2. *Lactobacillus acidophilus* was identified after the genomic sequencing with ascribed NCBI accession number of JX255677 as shown in the evolutionary relatedness tress (Table 1 and Figure 3). Strains of *Lactobacilli acidophilus* isolated from the dairy effluent showed the ability to produce β-galactosidase when screened with chromogenic substrates (p-NPG and o-NPG) as shown in Figure 1A. Formation of yellow colouration in the nutrient broth after 48 hours of incubation at 37°C suggests a positive test for β-galactosidase production. Gheytanchi et al. reported similar observation, working with *Lactobacillus* from milk and cheese using o-NPG as their standard screening substrate [10].

Studies on the effect of incubation period on microbial growth rates at different inoculum sizes showed maximum microbial growth of $\times 10^8$ CFU/ml in all the inoculum sizes after 36 hours of incubation (Figure 4). Increase in the inoculums size was accompanied proportionately with increase in microbial growth. Decline in microbial growth was observed after 36 hours could be attributed to over multiplicity of the microbes leading to deficient nutrient in the media [9].

Studies on effect of incubation period on the production of β-galactosidase from *Lactobacilli sp*. showed that the highest β-galactosidase activity and protein concentration were obtained on the day 12th and 13th of the fermentation. Extracellular protein production at these designated days respectively is evident of catabolite inducement of the substrate present in the fermentation media to the organisms. Closeness in the optimal β-galactosidase activity and protein concentration is unique to most extracellular protein. Akcan reported day 8 as the peak production day for β-galactosidase from *Bacillus licheniformis* ATCC-12759 in submerged fermentation system [8].

pH is an important physiologic factor to be considered in enzyme production system. It helps to understand the tolerant nature of the organisms to hydrogen ion (H+) concentration of the medium. The optimum pH for β-galactosidase production was 6.0 (Figure 5). Lactic acid bacteria are moderate acidophiles and are tolerant to low pH in production system. The report by Gheytanchi et al. on β-galactosidase produced from milk and cheese showed a production pH of 6.5 and 5.5 respectively [10]. Effect of Nitrogen (nutrient) sources on β-galactosidase production from *Lactobacilli acidophilus* showed that peptone extract was the best nitrogen for β-galactosidase production (Figure 7). As reported by Vasiljevic and Jelen, organic nitrogen sources in fermentation media are super source of amino groups to fermenters in bio-production process [14].

Also, studies on the effect of carbon sources on production of β-galactosidase from *Lactobacilli acidophilus* showed that lactose was the best for β-galactosidase production followed by sugar cane baggase. The preference for lactose by the organism for the enzyme production (β-galactosidase) is evident as lactose plays a good catabolite activator for exo β-galactosidase production in the Lac operon as described by Monod and Jacob [2]. Lactose, a disaccharide of glucose and galactose is a sole inducer of organisms for exo-secretion of β -galactosidase in any medium. Ahmed et al. reported lactose from dairy whey as the best source of the sugar for the production of β-galactosidase from *Lactobacillus acidophilus* [15].

## CONCLUSION

Bacteria, fungus, yeast, plants, and recombinant sources have all been used to isolate the enzyme -galactosidases. This enzyme is significant because of its many uses in the food industry, including the production of lactose-hydrolyzed goods for lactose-intolerant persons and the synthesis of glycosylated products. Lactose intolerance is caused by a lack of activity of this enzyme, which allows undigested lactose to be absorbed in the small intestine. Lactose intolerance affects over 70% of the world's adult population, with lactose intolerance being prevalent at 60%. Galactosidases are utilized in the production of lactose-free goods, as well as in the treatment of whey and the production of prebiotics. The relevance of galactosidase in the food industry is high which focuses on several sources of galactosidase. This research has demonstrated that *lactobacillus acidophilus* isolated from dairy effluent can be a potential and sustainable source of β-galactosidase which stands to be an integral part of evolving dairy and other confectionary industries.

## REFERENCES

1. Husain Q. β Galactosidases and their potential applications: A review. Crit Rev Biotechnol. 2010;30(1):41-62.
2. Cohn M, Monod J. Purification et propriétés de la β-galactosidase (lactase) d'Escherichia coli. Biochim Biophys Acta. 1951;7:153-174.
3. Chen W, Chen H, Xia Y, Zhao J, Tian F, Zhang H. Production, purification, and characterization of a potential thermostable galactosidase for milk lactose hydrolysis from Bacillus stearothermophilus. J Dairy Sci. 2008;91(5):1751-1758.
4. Wyckoff HW, Doscher M, Tsernoglou D, Inagami T, Johnson LN, Hardman KD,et al. Design of a diffractometer and flow cell system for X-ray analysis of crystalline proteins with applications to the crystal chemistry of ribonuclease-S. J mol bio.1967;27(3):563-578.
5. Tari C, Ustok FI, Harsa S. Production of food grade β-galactosidase from artisanal yogurt strains. Food Biotechnol. 2010;9:24(1):78-94.
6. Sinnott ML, Withers SG. The β-galactosidase-catalysed hydrolyses of β-d-galactopyranosyl pyridinium salts. Rate-limiting generation of an enzyme-bound galactopyranosyl cation in a process dependent only on aglycone acidity.J Biochem. 1974;143(3):751-62.
7. Bras NF, Fernandes PA, Ramos MJ. QM/MM studies on the β-galactosidase catalytic mechanism: Hydrolysis and transglycosylation reactions. Journal of chemical theory and computation. 2010;6(2): 421-33.
8. Akcan N. High level production of extracellular-galactosidase from Bacillus licheniformis ATCC 12759 in submerged fermentation. Afr J Microbio Res. 2011;5(26):4615-21.
9. Ezeonu M, Okafor J, Ogbonna J. Laboratory Exercises in Microbiology.1st edn. Ephrata Publishing and Printing Company, Nsukka. 2013:100-117
10. Gheytanchi E, Heshmati F, Shargh BK, Nowroozi J, Movahedzadeh F. Study on b-galactosidase enzyme produced by isolated *Lactobacilli* from milk and cheese. Afr J Microbiol Res. 2010;4(6):454-8.
11. Chilaka F, Nwachukwu A, Uvere P. Thermal stability studies of β-galactosidase from germinating seeds of the brown beans, vigna unguiculata. Nig J Biochem Mol Biol. 2002;17(1): 51-56.
12. Lowry O, Roseburg N, Farr A, Randall R. (1951) Protein Measurement with Folin- Phenol Reagents. J Bio Chem. 93:265-275.
13. Allam R, Aly M, El-zhrany K, Shafei, M. Production of β-Galactosidase Enzyme from *Lactobacillus acidophilus* RK isolated from different sources of milk and dairy products. Int J ChemTech Res. 2016; 9(10):218-231.
14. Vasiljevic T, Jelen P. Production of β-galactosidase for lactose hydrolysis in milk and dairy products using thermophilic lactic acid bacteria. Innov Food Sci Emerg Technol. 2001;2(2):75-85.
15. Ahmed S, lingappa k, Divatar M, Gajare S, Shivalee A, Farheen A, et al. Optimization of lactase production under submerged fermentation by *Lactobacillus sp.* KLSA 22. Int J Pure Appl. 2016;4:212-20.