PRODUCTION AND PARTIAL PURIFICATION OF BETA-GALACTOSIDASE PRODUCED BY CANDIDA TROPICALIS ISOLATED FROM DAIRY EFFLUENT

[1]*Maikaji F.S., [2]Hauwa, H. and [1]Ibrahim A.A.

[1]National Animal Production Research Institute (NAPRI), Zaria
[2]Sokoto State University, Sokoto, Nigeria
*Corresponding author: fmaikaji@gmail.com; 07035040300

ABSTRACT
Beta-galactosidase (-D-galactoside galactohydrolyse; E.C. 3.2.1.23), in other words lactase, is a commercially important enzyme that catalyzes the hydrolysis of lactose into its constituent monosaccharides glucose and galactose. It is widely distributed in nature, found in numerous plants, animals and microorganisms including yeast, fungi and bacteria. Hence, the study was designed to isolate, identify, optimization of production conditions for maximal production of β-galactosidase, extraction and partial purification of the enzyme. The proximate composition of the dairy effluent were determined using AOAC methods and it revealed; moisture (80%), ash (0.160%), crude fat (5.57%), crude protein (0.88%) and Carbohydrate (13.21%). The isolated yeast strains were found to metabolize and ferment various mono and disaccharides which includes glucose, maltose, lactose, sucrose, and raffinose. Analytical Profile Index kit (API) was further used to confirm the yeast isolates Candida tropicalis. The activity of the enzyme was higher after 48 hrs fermentation period (0.089U/mL) during optimization. The enzyme displayed maximal activity around neutral pH of 6.0 (0.075U/mL) and at the temperature of 30°C (0.076U/mL). The Beta galactosidase was partially purified through Ammonium sulphate precipitation (75% saturation), Gel filtration and Ion exchange chromatography. The yield obtained after purification was (89.1%) with purification fold of (1.14) and specific activity of (0.224U/mg). Therefore, dairy effluent could serve as a cheap and efficient substrate for large scale production of Beta-galactosidase.
Keywords: Beta-galactosidase, Dairy effluent, *Candida tropicalis*

INTRODUCTION

Enzymes are the most essential bio molecules obtained from different microbial sources such as bacteria and fungi for human needs (Najish *et al*., 2016). They control and speed up the chemical reactions that's take place inside the living organism. Enzymes are used to make foodstuffs, pre- digest some baby foods, extract fruit juices, in biological detergent and as in biosensors (Mozumder *et al.*,2012). Currently the field of biotechnology is developing new applications for enzymes.

Beta-galactosidase also known as Beta-gal, is an extracellular and hydrolytic enzyme that catalyses the breakdown of lactose (disaccharide in milk) into galactose and glucose which are monosacharides. β-galactosidase is also involved in transglycosylation reaction. β-galactosidase is widely distributed in animals, plants and numerous microorganisms such as, bacteria, fungi, archaea and yeast (Najish *et al*., 2016). β-galactosidase has two main uses; the production of galactosylated products and the lactose removal from milk products for lactose intolerant people.

Beta-galactosidases are commercially produced from yeast such as *Kluyveromyces lactis* and *Kluyveromyces marxianus,* and moulds such as *Aspergillus niger* and *Aspergillus oryzae* (Kumari *et al.*,2011). Bacterial species such as *Bacillus coagulans, Bacillus circulans, Escherichia coli, Lactobacillus bulgaricus, Lactobacillus thermophile* also produce β galactosidase (Mozumder *et al*., 2012). It is important to select microorganism which produce β galactosidase with high catalytic efficiency (Mlichov and Rosenberg 2006). The lactose reduced ingredients in the food and dairy products are commercially produced for lactose intolerant persons (Rajakala and Selvi, 2006). Microorganisms offer various advantages over other available sources such as ease of handling, higher multiplication rate, high activities of the enzyme, good stability 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 (Rajakala and Selvi, 2006).

The β-galactosidase assay is used frequently in genetics, molecular biology and other life sciences*(Krivtsov and Armstrong, 2007)*.The enzyme also has many industrial and medicinal applications like cleavage of blood group A and B glycotopes, biosensors for lactose determination and enzymatic hydrolysis of lactose (Asraf and Gunasekaran, 2010). Beta-galactosidase (EC 3.3.1.23) is one of the most important enzymes used in the food industry, which offers nutritional, technological and environmental applications (Torres *et al*., 2010). Hydrolytic activity of this enzyme is important for applications in the food industry in reducing the lactose content in milk, and increases the solubility and sweetness in dairy products (Otieno, 2010). Beta-galactosidase is used in such dairy products as yoghurt, sour cream, and some cheeses which are treated with the enzyme to break down any lactose before human consumption.

Collection of Sample

The dairy effluent was collected from the Processing Unit of the Dairy Research Programme of National Animal Production Research Institute (NAPRI) Shika, Ahmadu Bello University, Zaria. The effluent sample was collected in clean Jerrycan and transported immediately to Department of Microbiology, Ahmadu Bello University Zaria. The sample was stored in the refrigerator for further analyses.

Proximate analysis of the Sterilized Dairy Effluent

The raw dairy effluent was filtered using a muslin cloth. Sterilization of the filtered dairy effluent was carried out by autoclaving at 121^0C for 15min. The proximate composition of the sterilized dairy effluent determined includes moisture content, ash content, crude fat, crude protein and carbohydrate content as described by Association of Official Analytical Chemists (AOAC,2010). Analyses were carried out in duplicates.

Isolation and characterization of Yeast

Yeast were isolated, characterized using the method of (Nizamuddin *et al,* 2008). The Analytical Profile Index kit was used to further confirm the isolates. The kit was used according to the manufacturer's guideline.

Production of Beta-galactosidase under Submerged Fermentation using Dairy Effluent as Substrate

Inoculum preparation

The selected isolate was sub cultured on Sabarauds Dextrose Agar (SDA) slants and incubated at room temperature for 5days. The grown yeast was diluted to concentration of 3.0 x 10^8 cells per ml (McFaland turbidity standard) scale No.2. This was used as inoculum for the fermentation experiment (Antoine *et al*., 2015).

Production of beta-galactosidase

Sterile dairy effluent was used as production medium. Using a sterile syringe, 2.5mL of the inoculum was aseptically inoculated into 500mL Erlenmeyer flask containing 250mL of the sterile production medium i.e. sterile dairy effluent. The broth was incubated at room temperature for 7days (Akinola *et al*., 2012). After incubation period, the broth was filtered through a double layered muslin cloth and the filtrate was centrifuged at 10,000 rpm for 10minutes at 4^oC. The supernatant was separated from the pellet using a clean Pasteur pipette into clean test tubes. The solution was then tested for beta-galactosidase activity (Murugan, 2013).

Determination of the effect physico-chemical parameter on Beta galactosidase production

The effect of Temperature at (25, 30, 35 and 40^0C for 7days), Incubation time (at optimum temperature 30^0C for 7days.) and pH (pH 5, 6, 7 and 8, and incubated at optimum temperature 30^0C and incubation time 48hrs)) were determined.

Partial purification of crude Beta galactosidase

The Beta-galactosidase produced was purified using *Ammonium sulphate precipitation*.(Seidman and Moore 2005), *Gel filtration chromatography* (Maria *et al*., 2006) and Ion exchange chromatography was carried out using Diethylaminoethyl (DEAE) as ion exchange resin (Somyos and Phimchanok, 2009).

RESULT AND DISCUSSION

Proximate composition of Treated Dairy Effluent

The color of the dairy effluent after autoclaving was observed to be white. The proximate composition of the treated dairy effluent revealed the moisture content of 2.71%, while the ash content 0.35%. The lipid content was 1.4% while the protein and carbohydrate contents were 2.24% and 0.30% respectively (Table 1).

Table 1: Proximate Composition of Raw Dairy Effluent

Parameter	Composition (%)
Moisture	80.0
Ash	0.160
Crude Fat	5.75
Crude Protein	0.88
Carbohydrate	13.21

Isolation and Characterization of Yeast Isolates from Dairy Effluents

A total of eight (8) yeasts were isolated from the raw dairy effluent. All Yeast isolates were obtained in pure cultures. The results of the macroscopic (cultural) and microscopic examination confirmed the isolates to be yeast. All the isolates were smooth, round, creamy and spherical in shape. The isolates were further confirmed using Analytical Profile Index kit to be; *Candida tropicalis*.

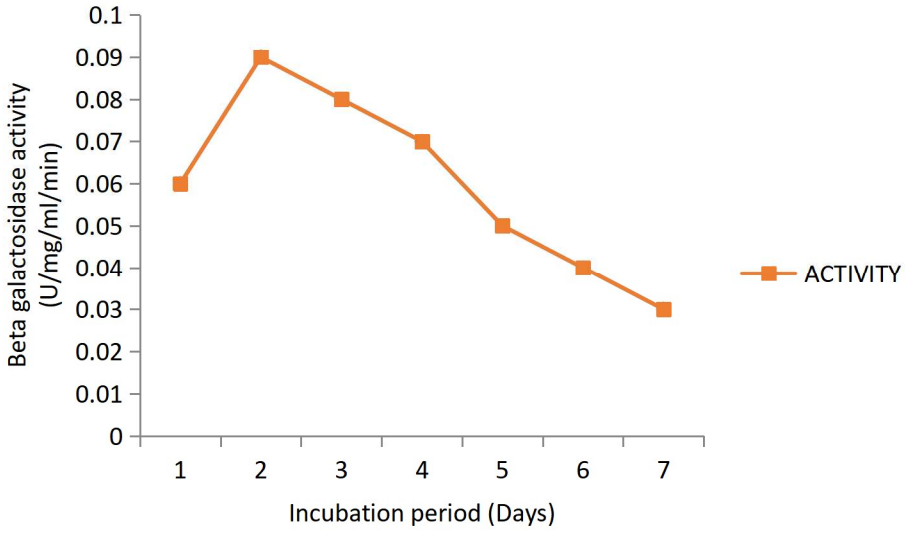

Fig 1: Effect of incubation time on Betagalactosidase production by *Candida tropicalis*

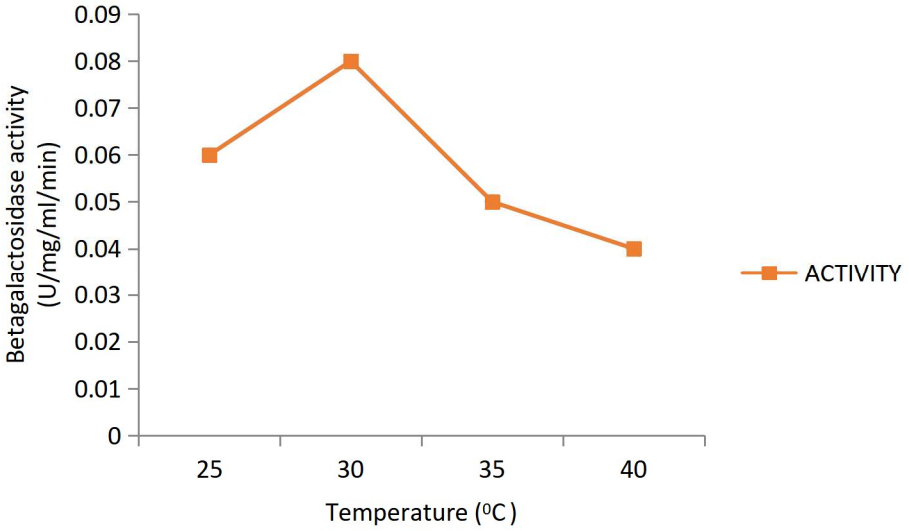

Fig 2: Effect of incubation temperature on Betagalactosidase production by *Candida tropicalis*

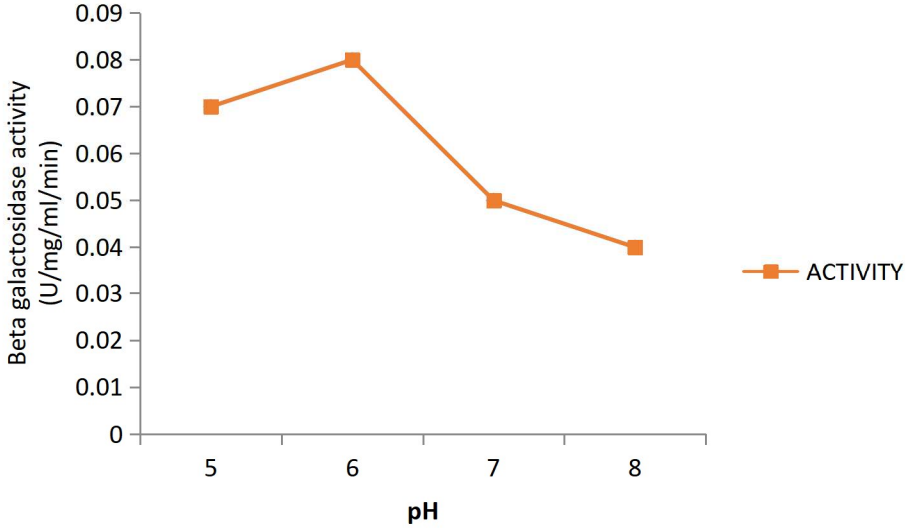

Fig. 3: Effect of initial pH on Betagalactosidase production by *Candida tropicalis*

Partial purification of β-Galactosidase produced by *C.tropicalis*

Purification Steps	Total activity(U/mL)	Total protein(mg)	Specific activity(U/mg)	Purification fold	Yield (%)
Crude beta-galactosidase	5.612	28.54	0.197	1	100
Ammonium sulphate precipitation (75%)	5.270	25.50	0.207	1.05	93.9
Gel filtration Chromatography	5.020	23.40	0.215	1.09	89.5
Ion exchange Chromatography	5.001	22.37	0.224	1.14	89.1

DISCUSSION

The result of the proximate composition reveals the moisture content of the dairy effluent to be 80.0%. This could be attributed to high amount of water used in washing the equipment in the dairy industries. The ash content was found to be 0.160%, which is an indication that the mineral content of the effluent is low. This could be attributed to the fact that less than 2% of total milk processed is wasted into drains (Munavalli and Saler, 2009). Hence, the mineral content from the less than 2% lost will be significantly low. The crude fat and crude protein was recorded as 5.75% and 0.88% respectively. The low fat and protein may be due to lipase and protease enzymes respectively present in detergents used to wash residual milk or milk products from the basins and other utensils. Carbohydrate was found to be 13.21%. This could be attributed to lack of accumulation of residual milk from the wastewater introduced into the drains (effluent was freshly collected). The major carbohydrate found in milk is lactose (Mahoney, 2005).A consequence of the presence of lactose in this amount is that the sterile dairy effluent could be a potential source of substrate for production of beta-galactosidase. This result agrees with studies carried out by Anvari and Khayati (2011) in Iran and Kaur *et al*., (2015) in India. Anvari and Khayati (2011) reported lactose content of 4.9%, 0.9% protein content and 0.05% fat content. Kaur *et al*., (2015) reported lactose content 5.00%, 0.48% protein content, 0.18% fat content and trace amount of mineral content in whey. This finding disagrees with the work of Victor *et al*., (2014) who reported 425g/L carbohydrate content, 7% protein content and 3.79% ash. This could be due to the difference in the nature of the sample analyzed or difference in methodology used.

The activity (0.101U/mL) of enzyme produced by *C. tropicalis* shows that the organism could utilized dairy effluent as substrate to produced beta-galactosidase. A consequence of this finding is that the sterile dairy effluent has a potential to be used as an alternative substrate for production of this enzymes with possible need for supplementation. This agreed with the work of Panaser *et al*. (2016) who got similar range of beta-galactosidase activity (0.022-2.14U/mL) produced by the variety of fungi isolated from various food and agro-industrial wastes using lactose broth as production media. This disagreed with the work of Vishwanataha *et al.* (2012) who found extracellular and intracellular betagalactosidase activities of 2250IU and 2477IU respectively from *Rhizopurs stolanifera* strain incubated with 2% lactose for 18hours. This disagreement could be due to the methodology, organism or substrate used for the enzyme production.

Under optimized incubation period yeasts produce higher amount of enzyme. As per Fig. 1 maximum enzyme activity of 0.089U/mL was observed after 48 hrs of incubation during fermentation. Betagalactosidase production decrease with an increasing time. This is due to decrease in metabolic activities of yeast cells from stationary to lag growth phase (decline phase) which may be caused by depletion of nutrient in the culture medium. Rech and Ayub (2007) attributed this to reduction to oxygen limitation caused by high oxidative metabolism of yeast

The maximum enzyme activity of 0.075U/mL was observed at pH 6.0. Above this value (6.0), the microbial growth and beta-galactosidase production are reduced. In this case, the pH of the medium affects generally RNA and protein synthesis, the functions of enzymes and the of nutrient in to the cell (Gupte and Nair, 2010). This agrees with the work of Manjusha and Khushbu (2015) who also reported pH 6.0 while producing beta-galactosidase from yeast spp.

Ammonium sulphate precipitation method for enzyme saturation was carried out at 35-75% for precipitation of crude enzyme. The result shows that at 75% of saturation with ammonium sulphate precipitated the enzyme under optimum condition. The total and specific activities of the partially purified beta galactosidase were 5.001U/mL and 0.224U/mg respectively. Batra *et al.,* (2002) used the same method to purify beta galactosidase produced by *Bacillus* species also reported a decrease in the total activity and increase in the specific activity of

16,723.3U and 1429.3U/mg. This may be due to elimination of impurities and removal of unwanted proteins during the purification steps.

CONCLUSION

The treated dairy effluent was high in moisture and considerably low in ash, carbohydrate, lipid and protein contents.

The beta galactosidase producing potential of *Candida tropicalis* was achieved and the optimum cultural conditions were found to be; temperature of 30^0C, initial pH of 6, and incubation time of 48hours.

The purification of *C.tropicalis* beta-galactosidase gave higher percentage yield of 89.1%, the total protein of 22.37mg, specific activity of 0.224U/mg and higher purification fold of 1.14.

REFERENCE

Anvari, M. and Khayati, G. (2011). Submerged Yeast Fermentation of Cheese Whey for Protein Production and Nutritional Profile Analysis. *Advance Journal of Food Science and Technology* ,3(2):122-126.

Asraf, S. S. and Gunasekaran, P. (2010). Current Trends of β-galactosidase Research and Application. In:Mendez-vilasa,Editor. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*. 2. Spain:Formatex Research Center.pp.880–889.

Association of Official Analytical Chemists (AOAC) (2010).Official Methods of Analysis of the Association of Analytical Chemist, (18thEdition).Wasington D.C. Association of Analytical Chemists.

Batra, N., Singh, J., Banerjee, U.C., Patnaik, P.R., 2002. " Production and characterization of a thermostable -galactosidase from Bacillus coagulans RCS3" *Biotechnol. Appl. Biochem.* Vol.36, p.1-6

Gupte A.M. and Nair J.S. (2010). Beta-galactosidase production and bioethanol fermentation from whey using Kluveromyces marxianus NCIM 355. *Journal of science and Industrial research* 69(11):855-859.

Krivtsov, A. V. and Armstrong S. A. (2007): MLL Translocations, Histone Modifications and Leukaemia Stem-Cell Development".Nature Reviews Cancer 7 (11): 823–833.

Kumari, S. Panesar, S. P. and Panesar, R. (2011). Production of β- Galactosidase using novel yeast isolate from whey, *International Journal of Dairy Science* Vol.6(2), pp. 150-157.

Mahoney, R. R. (2005). Modification of Lactose and Lactose-Containing Dairy Products with Beta-Galactosidase.In: Developments in Dairy Chemistry, Elsevier,England.69– 110.

Manjusha, S. D. and Khushbu, G. (2015) Isolation of β-galactosidase from a Yeast sp. Isolated from whey. *International Journal of Advanced Biotechnology and Research* (IJBR) Vol 6, Issue 3, 2015, pp425-432.

Mlichov, Z. and Rosenberg, M. (2006). Current trends of β-galactosidase application in food technology, *Journal of Food and Nutrition Research,* Vol.45, pp. 47-54.

Mozumder, N. H. M. R., Akhtaruzzaman, A. Bakr, M. A. and Tuj-Zohra, F. (2012). Study on Isolation and Partial Purification of Lactase (β-Galactosidase) Enzyme from *Lactobacillus* Bacteria Isolated from Yogurt, *Journal Scientific Research,* Vol.4 (1), pp. 239- 249.

Munavalli, G. R. and Saler, P. S. (2009). Treatment of Dairy Waste Water by Water Hyacinth. *Water Science Technology*,59(4):713–722.

Murugan, T. (2013). Isolation, Screening and Characterization of Beta-Galactosidase Enzyme ProducingMicroorganisms from Four Different Samples. *Global Research Journal of pharmaceutical sciences.*013 2(1):12-14

Najish, M. A., Saiqa, A., Bushra, M. and Agha, U. A. (2016) Optimization of β-galactosidase Production from Yogurt and Dairy Soil Associated Bacteria Using Different Fermentation Media, *British Microbiology Research Journal* 11(2): pp 1-15.

Nizamuddin, S. Sridevi, A. and Narasimha, G. (2008) Production of β-galactosidase by *Aspergillus oryzae* in solid-state fermentation. *African Journal of Biotechnology* Vol. 7 (8), pp. 1096-110

Otieno, D. O. (2010). *Food Science and Food Safety* 9:471—482.

Rajakala, P. and Selvi, P. K. (2006). The effect of pH, temperature and alkali metal ions on the hydrolsis of whey lactose catalysed by β- galactosidase from *Kluyveromyces marxianus. International Journal of Dairy Science.* 1: 167-172.

Rech R. and Ayub M. (2007). Simplified feeding strategies for fed-batch cultivation of *Kluveromyces marxianus* in cheese whey. *Process Biochemistry*, Vol 42, pages 873-877.

Seidman, L. A. Moore, C .J. (2005). *Basic laboratory methods for biotechnology.* Prentice Hall, New Jersey

Somyos, O. and Phimchanok, J. (2009). Isolation and Characterization of Beta-Galactosidase from Thermophile B1. *Asian Journal of Food and Agro-Industry* ISSN19063040.www.ajofai.info. 135-143.

Torres, D.P.M., Goncalves M.P.F., Teixeira J.A., Rodrigues L.R. (2010). *Food Science and Food Safety* 9: 438—454.

Victor, U. Ashok, K. B. Cornish, K. and Ezeji, C. (2014). Evaluation of Industrial Waste (Milk Dust Powder) for Acetone-Butanol-Ethanol Production by Solventogenic Clostridium species. *Springerplus* ,3:387.

Vishwanataha, T. Sampath, A. Spoorthi, N. J. Divyashree, B. C. Reena, V. Sowmya, G. Mohan, K. B. S. Venugopal, N. Sharangowda, J. P. and Siddalingeshwara, K. (2012). A Novel Approach for Screening and Synthesis of β-Galactosidase from Microbial Origin. International Journal of Applied Biotechnology and Biochemistry, 2(3):285-290