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PRODUCTION OPTIMIZATION, PURIFICATION AND CHARACTERIZATION OF THE A-GALACTOSIDASE FROM *FUSARIUM MONILIFORME* NCIM 1099

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ABSTRACT: The α-galactosidase from Fusarium moniliforme is well optimized for various physico-chemical parameters such as pH, temperature, buffer molarity and also effect of different carbon and nitrogen sources were studied in order to determine the optimum conditions for the production of α- galactosidase. The α-galactosidase production by the fungal strain cultivated with shaking condition was found to be more suitable than stationary conditions. The media optimization with one-factor-at-a-time experiment reveals, galactose (1.105U/mg) and melibiose (1.032U/mg) were found to be the carbon sources. Among the nitrogen sources, soya peptone (0.903U/mg), peptone (0.801U/mg), soybean meal (0.711U/mg) followed by urea (0.607U/mg) were shown to serve well. The effect of various mineral salts also well accounted here.The partial purification carried out with 70 % ammonium sulphate saturation showed good precipitation and 2-3 fold more activity than the other organic solvent precipitation. The used purification procedure provided 3.95 fold purification with 14.48 % yield. The enzyme showed optimal α-galactosidase activity with the citrate-phosphate buffer molarity of 20mM/l having pH 5.0 and with temperature of 50°C.

KEYWORDS: *Fusarium moniliforme*, α-galactosidase, carbon sources, nitrogen sources, optimization purification

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1.INTRODUCTION

α-Galactosidase (α-D-galactoside galactohydrolase EC (3.2.1.22) is widely distributed in microorganisms, plants, and animals. A novel α-galactosidase was identified from the hyperthermophile archaeon Sulfolobus solfataricus [1]. These, glycosidases or carbohydrolases are reported for playing pivotal role in hydrolysis of carbohydrates. It is an exoglycosidase that catalyses hydrolysis of terminal α-1, 6 linked galactosyl residue from a wide range of substrates including, oligosaccharides of raffinose family sugars such as raffinose, stachyose, melibiose, verbascose and polysaccharides of galactomannans, locust bean and guar gum. Cellular localization and purification was studied in variety of microorganisms such as Saccharomyces carlbergensis [2], Pichia gulliermandii [3], Candida javanica [4], Aureobasidium pullulans [5] etc. It hydrolyzes a variety of simple α-D-galactoside as well as more complex molecules such as oligosaccharides and polysaccharides and is also having number of biotechnological and clinical applications like production of sugar, processing of soymilk, conversion of blood type; type B erythrocytes which contain 3-O-α-D-galactopyranoside, this can be transformed into type O erythrocytes after exposure to α-galactosidase [6] and in the treatment of Fabry's disease respectively [7]. Because of its immense commercial applications it is used in different industries like beet sugar, in removing of raffinose from molasses and sugar syrups etc. [8]. The enzyme is applied to increase the nutritional quality of legumes by hydrolyzing galactooligosaccharides and to increase gelling in guar gum [9]. Human consumption of soy-derived products has been restricted due to presence of α-galactosides; mostly raffinose and stachyose in soybeans. [10]. Although the use of microbial α-galactosidase appears to be a promising solution for the degradation of these non- digestible oligosaccharides in soy products [11]. There is considerable interest in the enzymes that catalyzes hydrolysis of glycosidic bonds, due to their extensive industrial, therapeutic and biochemical applications. Immobilization and its application in hydrolysis of raffinose in beet sugar molasses of thermostable α-galactosidase from Pycnoporus cinnabarinus was studied by using chitosan beads [12]. The aim of the present research work is to assess the proficiency of fungal strain Fusarium monoliforme for the production of α-galactosidase and its optimisation for the maximum enzyme production as well as the activity. The work will be much more beneficial for the increase in the existing knowledge about the study enzyme as well as about the knowledge of biochemistry.

2. MATERIALS AND METHODS

2.1. Chemicals and media components

All the required chemicals like p-Nitrophenyl α-D-galactopyranoside (pnpg), p-Nitrophenol were obtained from Sigma Chemical Co., all other sugars and chemical reagents were of analytical grade and purchased from Himedia Co. and Local suppliers in India.

2.3. Organism maintenance and Growth Conditions

Fusarium moniliforme NCIM 1099 used in this study was obtained from National Collection of Industrial Microorganisms, NCL, Pune, (M.S.) India. The strain was cultured and maintained on potato dextrose agar (PDA).

2.3. Preparation of medium and inoculation of fungus for the production of α-galactosidase

The inoculum medium consisted of 50 mL potato dextrose broth in 250 mL conical flasks. 1% solution of the spores of 48 h old culture of the fungus was used to inoculate the basal medium containing (g/L) sucrose, 10; NaNO3, 3; K2HPO4, 1; MgSO4.7H2O, 0.5; KCL, 0.5; FeSO4 0.01. After incubation period of about 96 hours, enzyme activity was assessed.

2.4. Optimization of different media parameters for enzyme production

Fusarium moniliforme was cultured in basal media broth at room temperature in a shaker at 120 rpm. The culture was harvested after every 24 hours interval, the mycelia were separated from culture broth by filtration through Whatman filter paper No.1, the filtrate was centrifuged at 8000 rpm for 15 min at 4°C and the clear supernatant was used for extracellular enzyme activity assessment. The harvested mycelia washed with 20 mM citrate-phosphate buffer (pH7) and homogenized using same buffer. The homogenate was allowed to stand for 15 min in the ice cold condition, filtered through Whatman filter paper No.1 and centrifuged at 8000 rpm for 15 min at 4°C and the clear supernatant was used to measure intracellular enzyme activity [13]. To screen out the optimal media components for α-galactosidase production, the nutrient components for production medium were varied as one factor at a time while keeping the others constant [14]. In this experimental set up, different carbon sources like glucose, galactose fructose, maltose, lactose, rhamnose, xylose, sucrose, melibiose and starch were used at 0.5% (w/v) as final concentration instead of sucrose from original basal medium [8] whereas the other parameters were unaltered and activity was assessed. To investigate the effect of different nitrogen sources on α-galactosidase production, original sodium nitrate from the basal medium was replaced with different organic (yeast extract, peptone, soya bean meal, soya peptone, urea) and inorganic (ammonium sulphate, ammonium chloride, potassium nitrate, sodium nitrate) compounds as a nitrogen source at 0.3% (w/v) as final concentration and by keeping rest of the media composition constant [15]. Effect of different essential components on α-galactosidase production was evaluated using essential minerals like FeSO4, (0.05%); CuSO4.5H2O, (0.05%); MgSO4.7H2O, (0.05%); NaH2PO4, (0.03%);and KH2PO4, (0.05%) [16].

2.5. Enzyme assay

Assay of a-galactosidase was carried out by the method of Dey and Pridham, with some modifications [17]. One milliliter of reaction mixture contains 0.1ml of enzyme solution, 0.8 ml of

0.2M Citrate phosphate buffer pH 5.0 and 0.1 ml of 1 mM p-nitrophenyl α-D galactopyranoside solution in distilled water. The mixture was incubated for 10 minutes at 50oC and then the reaction was arrested by the addition of 0.5 ml of 0.5M sodium carbonate solution. The quantity of p-nitro phenol liberated was measured by the absorbance at 405 nm. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 µmol of p-nitro phenol per min per ml of enzyme under assay conditions. The specific activity is expressed as units of enzyme activity per microgram of protein content in the enzyme source.

2.6. Partial purification of α-galactosidase

The activity of intracellular portion has observed to be maximum than the extracellular portion, the α-galactosidase enzyme from the homogenized cells was used for further purification studies. The crude enzyme was precipitated by using two methods for determining the proficient purification protocol.

2.6.1. Organic solvent precipitation

The centrifuged cell homogenate was subjected to precipitation by chilled acetone, methanol at 1:1 dilution at 4oC. The precipitate was collected by centrifugation at 6000rpm for 15 min at 4oC and dissolved in 0.02M citrate phosphate buffer of pH 7 and used for assessment of its enzyme activity.

2.6.2. Ammonium sulphate precipitation and dialysis

The above centrifuged cell homogenate was also subjected to precipitation by using ammonium sulphate at range of 10-80% of its saturation. The precipitate was collected by centrifugation at 8000 rpm for 15 min at 4oC, re-dissolved in 0.05M citrate phosphate buffer of pH 7, dialyzed against same buffer and used for assessment of its enzyme activity. In this method 10%- 80% ammonium sulphate was added in 10 ml of each centrifuged broth by continuous stirring at 4˚C till precipitation occurred. The precipitated material was subjected to centrifugation at 8000 rpm for 10 min at 4˚C to get precipitated pellet. The pellet was dissolved in 5 ml of 0.05M citrate phosphate buffer of pH 7andt he enzyme activity and protein concentration was determined by DNSA and Lowery method respectively. The sample was dialyzed against same buffer at 4^0C. Then the enzyme activity and protein concentration were determined as described previously**.**

2.6.3 Ion Exchange Chromatography

DEAE cellulose anion exchanger column was used for purification. The column had been pre-equilibrated with 0.1 M phosphate buffer. The dialyzed sample was loaded on column, the column was run with 0.1M phosphate buffer of pH 6.6 and elution fractions of 5 ml each were collected at flow rate 1 ml/min. The protein bound to the DEAE cellulose column were eluted using continuous salt gradients of 0.1M, 0.2M, 0.3M, 0.4M and 0.5M NaCl. The protein content of collected samples were detected by Lowery method. The active fractions containing higher protein content were

pooled for determination of enzyme activity by using DNSA method.

2.7. Protein estimation

Lowry method was utilized for the estimation of protein quantities in the enzymatic extract with bovine albumin serum as standard protein [18].

2.8. Characterization and optimization of Purified enzyme

2.8.1. Effect of pH on α- galactosidase activity

To estimate the optimum pH for α-Galactosidase activity the various buffers were used in varying pH range of 3 to 11 where citrate-phosphate buffer (for pH 3 to 6); phosphate buffer (for pH 7 to 8); Glycine-NaOH buffer (for pH 9 to 11); at 50°C were used.

2.8.2. Effect of temperature on α- galactosidase activity

The effect of temperature on the enzyme activity was evaluated using different incubation temperatures ranging from 10- 80ºC while keeping other physicochemical parameters unaltered.

2.8.3. Effect of buffer molarity on α- galactosidase activity

It is hypothesized here that the concentration of the buffer is important for the optimal enzyme activity, so bufferof varying concentration by means ofmolarity were used ranging from 10mM to 80mM concentrations.

2.8.4 Effect of different metal ions on enzyme activity

The effect of different metal ions such as Cu+, Hg2+, Mg+, Fe+, Ca+, Mn+, Na+ at 1mM concentration was evaluated while keeping other physicochemical parameters unaltered.

3. RESULTS AND DISCUSSION

3.1. α-galactosidase production

The 48 hrs growing fresh culture of Fusarium moniliformefrom potato dextrose broth was inoculated in 250 ml flask containing 100 ml production medium (basal medium with melibiose as carbon source). Two sets of experiments were conducted for static and shaking condition respectively. Significantactivity was observed in the shaking condition thanthe static one. Hence further experiments were conducted only with shaking condition. Localization of enzyme was studied by monitoringenzyme activity both intracellular as well as extracellular fractionsafter the interval of every 24 hours. The maximum α-galactosidase production was observed on sixth day of the incubation.

3.2. Production optimization of α-galactosidase enzyme

3.2.1. Effect of different carbon sources on α-galactosidase activity

Effect of different carbon sources on the growth of Fusarium moniliforme and activity of enzyme was depicted by calculating both intracellular and extracellular activity of α- galactosidase. We observed that galactose showed maximum intracellular activity as compared to other sugars whereas

xylose as sole carbon source shown maximum extracellular enzyme activity. When carbon sources were used with one variable at a time, significant enzyme production was obtained in the presence of galactose followed by melibiose. It seems that for the biosynthesis of α-galactosidase the presence of galactose and melibiose may serves as a best inducing environment while other sugars had a very little influence on enzyme induction.The data shows that the specificity of α-galactosidase is more for Galactose (1.105U/mg), melibiose (1.032U/mg) than for others. The effect of various carbon sources on α-galactosidase production is depicted in Fig.1

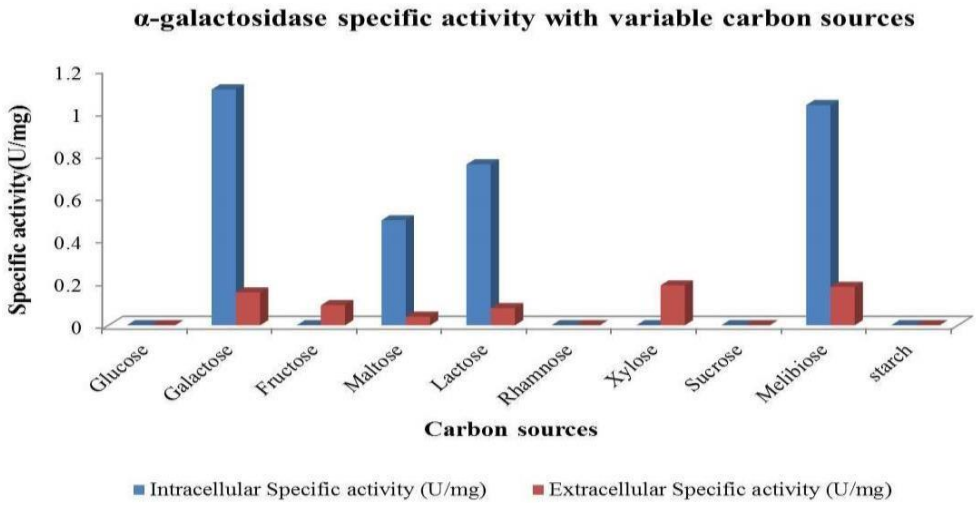

Fig.1 Effect of different carbon sources on intracellular and extracellular α-galactosidase specific activity

3.2.2. Effect of different nitrogen sources on α-galactosidase activity

Soya peptone shows induced α- galactosidase enzyme activity (0.903U/mg) followed by soybean meal (0.711U/mg) while other nitrogen sources have no significant effect on it. The results are well explained in the Fig.2

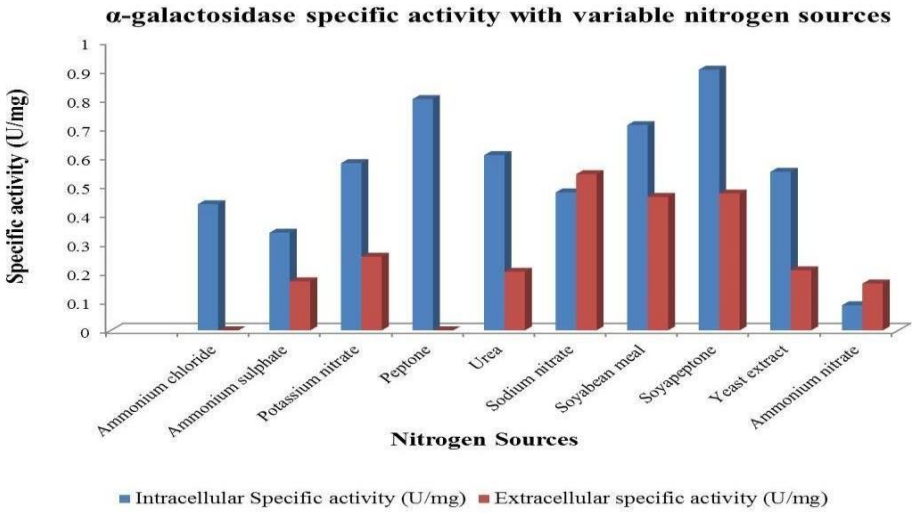

Fig.2 Effect of different nitrogen sources on intracellular and extracellular α-galactosidase specific activity

3.2.3. Effect of different essential mineral components on α-galactosidase activity

Among the essential minerals tested, FeSO4 induced a marked increase in α-galactosidase activity (0.454 U/ml) while CuSO4.5H2O slightly lowered enzyme production, whereas MgSO4.7H2O, NaH2PO4, KH2PO4 had no marked effect on enzyme production. The results are shown in the Fig.3.

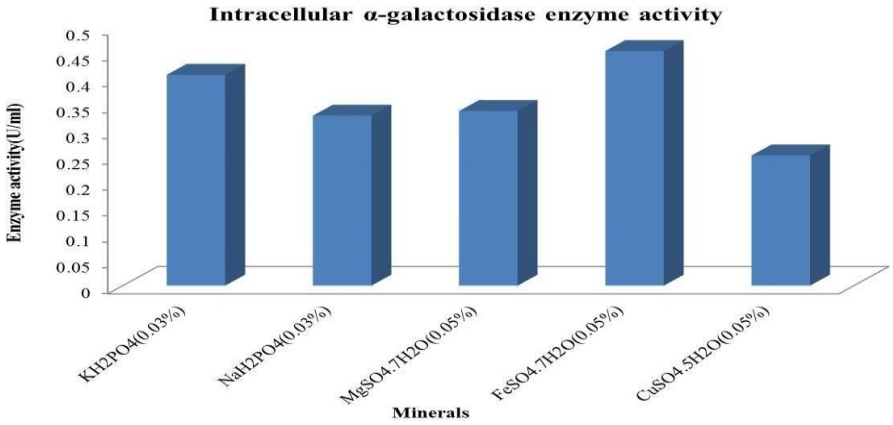

Fig.3 Effect of Minerals on Enzyme activity

3.4.3 Optimization of enzyme:

The enzyme was subjected to the various ranges of permutations and combinations of the assay conditions using acidic to alkaline range of pH and the temperature varying up line and down to room temperature.

3.5. Partial purification

The enzyme activity calculated by the precipitated enzyme using organic solvent as well as ammonium sulphate has compared in Table 1. It was observed that the enzyme precipitated at 70% ammonium sulphate has shown comparatively more activity than methanol & acetone precipitated.

Table.1 Comparison of the enzyme precipitation methods

Sr. No.	Precipitation done by	Enzyme activity (U/ml)
1	Methanol	0.169
2	Acetone	0.281
3	$(NH_4)_2SO_4$	0.679

3.4.2 Ion Exchange Chromatography:

Enzyme was partially purified by using DEAE cellulose, the elution profile of α- galactosidase (fig.4) showing maximum enzyme activity in fraction number 12 which has significantly increased the specific activity to 1.76 U/mg with 3.95 purification fold with 14.48% yield (Table 2).

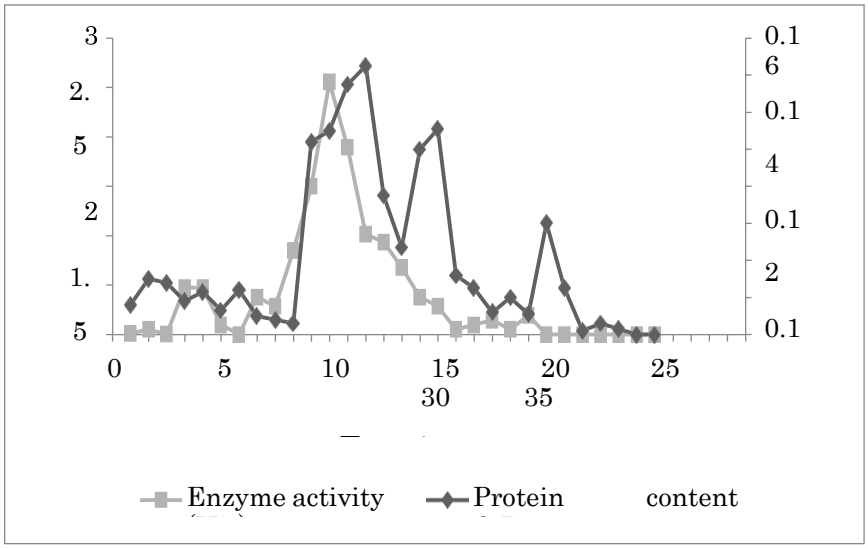

Fig. 4. Purification profile of α-galactosidase

The α-galactosidase from *Fusarium moniliforme* NCIM1099 was precipitated by 70% saturation of ammonium sulfate, desalted, concentrated and then loaded on DEAE cellulose DE52, anion exchange column chromatography (column diameter height = 1.2 cm X 18 cm). The proteins from column were eluted at 0.1–0.5 M NaCl gradient and the protein content was determined by taking absorbance at 280 nm, α-galactosidase activity was determined by standard assay method. The fraction size was of 5 ml each. The activity has eluted in fraction number 12 with 0.2 M NaCl.

Table.2 Purification of the α-galactosidase from *Fusariumm moniliforme* NCIM 1099

Enzyme	Fraction	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Yield
α-galactosidase	Crude	24.3	54.4	0.446691	1	100
	$(NH_4)_2SO_4$	6.79	4.54	1.495595	3.348163	27.94239
	IEC	3.521	1.995	1.764912	3.951079	14.48971

3.6. Characterization and optimization of purified enzyme

The α-galactosidase was observed to have an optimum enzyme activity at pH 5 with incubation temperature of 50°C. The concentration of the buffer was observed to influence the enzyme activity and the concentration of 20mM of citrate phosphate buffer showed maximum activity at optimized other conditions. The results are well illustrated in Fig.5, Fig.6 and Fig.7. The effect of different metal ions such as Cu+, Hg2+, Mg+, Fe+, Ca+, Mn+, and Na+ at 1mM concentration was evaluated. Among them Hg2+ shows inhibitory activity.

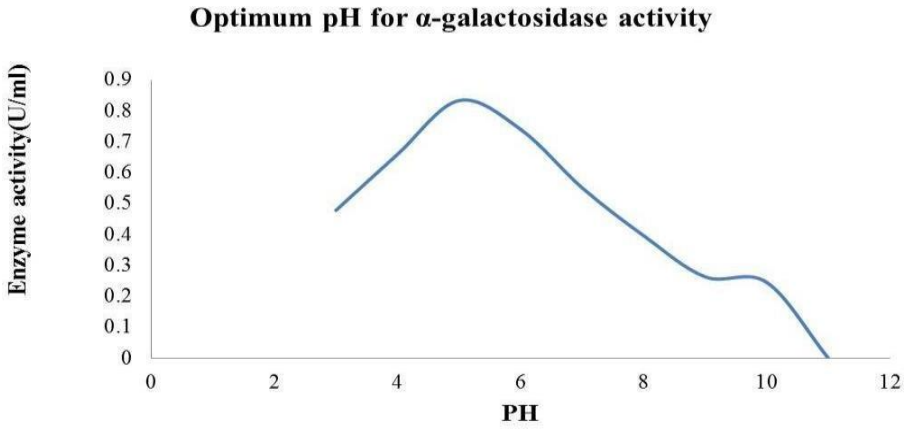

Fig.4 Effect of pH on α-galactosidase activity

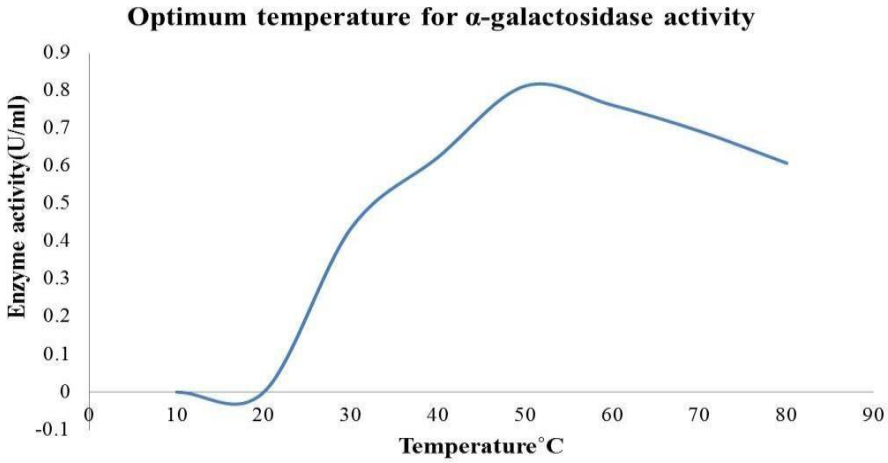

Fig.5 Effect of temperature on α-galactosidase activity

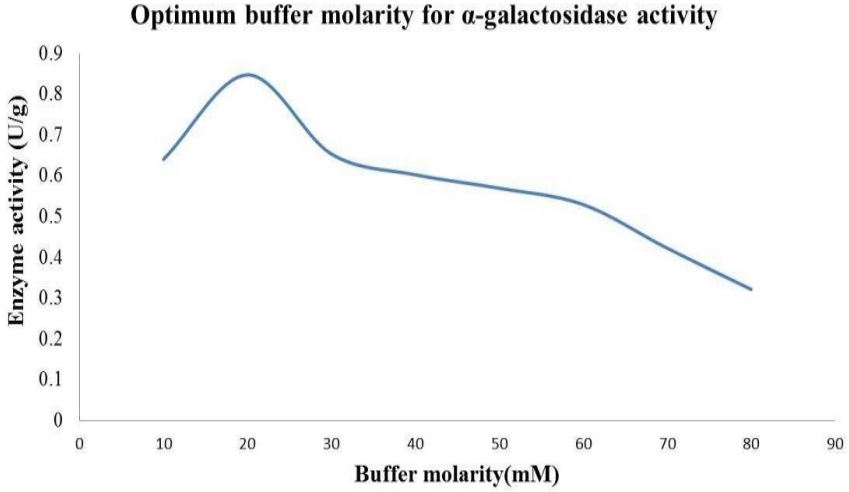

Fig.6 Effect of buffer molarity on enzyme activity

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4. CONCLUSION

Now days the production of industrially applicable microbial enzymes are thirst of research as these sources are more efficient, significant and cheaply available than the other sources such as plants, animals etc. Hence, the present research article emphasizes the production of α- galactosidase from the fungus *Fusarium moniliforme* NCIM No.1099. The present study deals with fungal species *Fusarium moniliforme* as a source of extracellular as well as intracellular α- galactosidase. The results obtained from the present research work suggest the *Fusarium moniliforme* NCIM No.1099 could serve as a potential fungal strain for production of α- galactosidase

REFERENCES

1. Brouns SJJ, Smits N, Wu H, Snijders APL, Wright PC, de Vos WM & Van der Oost J (2006) Identification of a novel α-galactosidase from the hyperthermophile archaeon Sulfolobus solfataricus. J Bacteriol 188:2392-2399.
2. Lazo PS, Ochoa AG & Gascón S (1977) alpha-Galactosidase from Saccharomyces carlsbergensis. Cellular localization and purification of the external enzyme. Eur J Biochem 77(2):375-382.
3. Church FC jr.1& Meyers SP (1980) alpha-galactosidase from Pichia Guilliermondii. .Mycologia 72(2):279-287.
4. Cavazzoni V, Adami A & Craveri R (1987) α-galactosidase from the yeast Candida javanica.Applmicrobiolbiotechnol26 (6):555-559.
5. Saha BC & Bothast RJ (1998) Purification and characterization of a Novel Thermostable a-L-Arabinofuranosidase from a Color-Variant Strain of Aureobasidium pullulans Appl Environ Microbio 64(1):216–220.
6. Tzortzis G, Jay A J, Baillon M L, Gibson G R, Rastall R A, (2003) Synthesis of α-galactooligosaccharides with α-galactosidase from Lactobacillus reuteri of canine origin, Appl Microbiol Biotechnol, 63: 286-292.
7. Fulle M, Lovejoy M, Brooks DA, Harkin ML, Hopwood JJ and Meikle PJ (2004). Immunoquantificatiion of alpha-galactosidase: evaluation for the diagnosis of Fabry disease ClinChem 50:1979-1985.
8. Sirisha E, Naveen A, Bharghav V & Lakshmi Narasu M (2010) Screening and isolation of intracellular α-galactosidase producing microorganisms from sugarcane waste soil. The Bioscan 5(4):579-582.
9. Bulpin PV, Gidley MJ, Jeffcoat R and Underwood DJ (1990) Development of biotechnological process for modification of galactomannan polymers with plant galactosidase. Carbohydrate Polymer 12: 155-168.
10. Thananunkul D, Tanaka M, Chichester CO and Lee T (1976) Degradation of raffinose and stachyose in soybean milk by α-galactosidase from Mortierella vinacea. Entrapment of α-galactosidase within polyacrylamide gel. Journal of Food Science 41:173-175.

11. Cruz R and Park YK (1982) Production of fungal α-galactosidase and its application to the hydrolysis of galacto- oligosaccharides in soybean milk. Journal of food Science 47:1973-1975.
12. Ohtakara A and Mitsutomi M (1987) Immobilization of thermostable α-galactosidase from Pycnoporus cinnabarinus on chitosan beads and its application to the hydrolysis of raffinose in beet sugar molasses. J Ferment Technol 65:493–498.
13. Siham Abdel-Azeem Ismail and Amal Mohamed Hashem (2012). Nutrition requirement for the production of Penicillium chrysogenum α-galactosidase and its potential for hydrolysis of raffinose family oligosaccharides. J Appl Sci Res 8(2):945-952.
14. Liu C, Ruan H, Shen H, Chen Q, Zhou B, Li Y, & He G (2007) Optimization of the fermentation medium for alpha-galactosidase production from AspergillusfoetidusZU-G1 using response surface methodology. J Food Sci 72:120-125.
15. Kumar Shivam, Chandra Prakash, Mani Tripathi, & Sarad Kumar Mishra (2009) Culture conditions for the production of α-galactosidase by Aspergillus parasiticus MTCC-2796: a novel source Electronic Journal of Biotechnology 12(4):1-9.
16. Lee J, Park I and ChoJ (2012) Production and partial characterization of α-galactosidase activity from an Antarctic bacterial isolate, Bacillus sp. LX-1.Afr J Biotechnol 11(60):12396-12405.
17. Dey P M, Wallenfels K (1974) Isolation and Characterization of α-galactosidase from Lens esculanta, Eur J Biochem, 50: 107-112.
18. Lowry OH, Rosebrough NJ, Farr AL & Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem193 (1):265–275.