The Bioscan 5(4) : 579-582, 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

SCREENING AND ISOLATION OF INTRACELLULAR α-GALACTOSIDASE PRODUCING MICROORGANISMS FROM SUGARCANE WASTE SOIL

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KEY WORDS
α-Galactosidase
Raffinose
Intracellular
Acinetobacter

Received on :
21.09.2010
Accepted on :
13.12.2010

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ABSTRACT
Intracellular α-galactosidase (EC 3.2.1.22) producing bacteria were isolated from soil. The isolates were grown on the growth media with sucrose as inducer. The isolate showing maximum activity was identified by following Bergey's manual. Different media parameters were optimized for enhanced enzyme production. α-galactosidase activity was optimal at 36ºC and pH 7.0 and when raffinose and tryptone were used as carbon and nitrogen source.

INTRODUCTION

Microbial enzymes are widely used in different industries such as food, beverage, textile, leather, pharmaceutical and waste water treatment. Of these, glycosidases or carbohydrolases plays a pivotal role in hydrolysis of carbohydrates. Glycosidases are classified into two categories-exoglycosidases and endoglycosidases based on the nature of hydrolysis. The exoglycosidases (ex: galactosidase and glucosidases) break the glycoside bond at terminal monosaccharide residue whereas endoglycosidases (Ex: amylases, endoglycosidase H) cleave the polysaccharide chains in between the monosaccharide residues. (Henrissat, 1991; Henrissat and Bairoch, 1993).

Galactosides are glucosides containing D-galactose linked to the aglycone molecule by acetal linkage. The enzymes that hydrolyse the D-galactose linkage are called galactosidases. Depending on the plane of hydrolysis of D-galactose molecules, galactosidases are classified into-α-galactosidase and β-galactosidases (Suzuki *et al*., 1970; Sinnott, 1990).

α-galactosidases (EC 3.2.1.22) catalyze the hydrolysis of α-1-6 linked terminal galactose residues from galactose oligosaccharides such as melibose, raffinose, stachyose and branched polysaccharides such as galactomannans and galactoglucomannans (Kumar and Mishra, 2010). α-galactosidases have variety of applications in medical and food industry. In beet sugar industry, this enzyme is used to remove raffinose from molasses, sugar syrups etc (Ohtakara and Mitsutomi, 1987). The enzyme is applied to increase the nutritional quality of legumes by hydrolysing galactooligosaccharides (Thippeswamy and Mulimani, 2002) and to increase gelling in guar gum (Buplin *et al.,* 1990). In humans, mutations in gfA gene leads to a X-linked lysosomal storage disorder called Fabry's disease. Enzyme replacement therapy with α-galactosidase is considered as potential treatment for Fabry's patients (Fulle *et al.,* 2004). In addition, the enzyme is applied for the conversion of type 'B' erythrocytes to type 'O' erythrocytes (Yang *et al*., 2000).

α- galactosidase has been isolated and purified from different sources like plants, plant seeds, yeasts, fungi and bacteria. Because of its immense commercial applications, the present study is focused on isolation of bacteria capable of α-galactosidase production from soils.

MATERIALS AND METHODS

Sample source

Soil samples were collected from sugar waste dumped soils from sugarcane industries located at Sangareddy (17º35' 07" N and 78º03' 50" E), Andhra Pradesh, India.

Isolation of microorganisms

The samples were serially diluted and plated on agar plates containing 1% (w/v) Sucrose, 1% (w/v) K_2HPO_4, 1% (w/v) Tryptone, 0.05% (w/v) Ascorbic acid, 0.01% (w/v) Thiamine HCl, 0.4% (w/v) $MgSO_4.7H_2O$, 0.2% (w/v) $FeSO_4.7H_2O$ (pH 7.0) hereby referred as growth media by spread plate technique. Plates were incubated at 37°C for 24 hr. Pure

cultures were obtained by using different pure culture techniques. The cultures were maintained on minimal medium agar slants containing sucrose, tryptone and K_2HPO_4 and subcultured every 15 days.

Screening of the isolates for α-galactosidase activity

Isolated colonies were inoculated into sucrose media and incubated at 36ºC for 24 hr in shaking incubator at agitation speed of 170rpm. Cells were harvested from broth by centrifugation at 10,000 g and washed with 20mM Tris buffer (pH 7.0). The cells were suspended in the same buffer containing 0.3% (w/v) lysozyme, 0.1% (w/v) Triton X 100, 1mM PMSF and incubated for 1 hr at 30ºC. The cells were further disrupted by sonication. Cell debris was removed by centrifugation (10,000 g, 20 minutes, 4ºC) and the supernatant was used for further study.

α-galactosidase assay

α - galactosidase activity was assayed in a reaction system containing 550μL of Tris buffer (pH 7.0), 100μL of enzyme preparation and 250μL of 2mm ρ-nitrophenyl-α-D-galactopyranoside (ρNPGal) or other synthetic substrates. The reaction mixture was incubated at 60ºC for 10 minutes and stopped by addition of 0.2mM Na_2CO_3 and read at 405 nm (Dey *et al.*, 1993). The activity was also measured by using raffinose as substrate in a reaction mixture containing 20mM tris buffer (pH 7.0) and enzyme preparation. The produced amount of reducing sugar was determined by adding 1 mL of 3, 5-dinitrosalicylate reagent (Miller, 1956).

One enzyme unit (U) was defined as the amount of enzyme required to produce one μmol of ρ-nitrophenol or reducing sugars (galactose) per min under the above assay conditions.

Protein estimation

Protein quantities in the enzymatic extract were determined by the (Lowry *et al.*, 1951) method with bovine albumin serum as standard

Culturing and characterization of the isolates

The isolate showing α-galactosidase activity hereby referred to as G2 was cultivated in the medium at 36ºC at 170rpm for 24 hr. Morphological and biochemical characteristics of the isolate have been studied for the identification of the isolate

Optimization of different media parameters for increased enzyme activity

Growth curve vs. Enzyme activity

G2 was cultured in media broth at 36ºC in a shaker at 170rpm. The culture was harvested at 2 hr interval. The cells were disrupted by above mentioned procedure and the supernatant collected was used as a crude enzyme solution for enzyme assay.

Effect of different carbon sources on α-galactosidase activity

The effect of different carbon sources on the growth of G2 has been studied by replacing the sole carbon source sucrose with glucose, raffinose, galactose, lactose, glucose + galactose at 1% (w/v) as final concentration whereas the other parameters were unaltered.

Effect of different nitrogen sources on α-galactosidase activity

Growth of G2 has been studied by using different nitrogen sources like yeast extract, tryptone, peptone, soyabean meal, yeast extract+ tryptone and casein at 1% (w/v) as final concentration, the other parameters were same.

Effect of pH on α-galactosidase activity

To observe effect of pH on the growth media for the production of α-galactosidase, pH of the media was varied from 5-9, the other parameters were unaltered.

Effect of temperature on α-galactosidase activity

For the selection of optimum temperature for the production of α-galactosidase the temperature was varied from 28ºC to 42ºC, by keeping the other parameters same.

RESULTS AND DISCUSSION

Microbiological analysis of soil samples revealed that sugar waste contaminated sites contain high bacterial count. 25 colonies were isolated and tested for α-galactosidase activity and one of the isolate showing maximum α-galactosidase activity was selected for further studies and was designated as G2.

Identification of the isolate

Morphological and biochemical characteristics of the isolate (G2) have been studied (Table 1) and the properties have been compared with the standard characteristics described in

Table 1: Morphological and biochemical characterisation of G2 isolate

Substrate, Test	Result
Gram Staining	Negative
Morphology	Coccobacilli
Citrate	Positive
Catalase	Positive
Gelatin liquefaction	Negative
Nitrate reduction	Positive
Oxidase	Negative
Fermentation of gas and acid	
Glucose	Positive
Sucrose	Positive
Mannose	Positive
Lactose	Negative
Indole	Negative
Methyl red	Positive
Voges-Proskauer	Negative

Table 2: Effect of different carbon sources on α-galactosidase activity

Carbon Source (1%)	Activity (IU)
Glucose	0.67
Galactose	2.24
Sucrose	3.85
Lactose	1.28
Raffinose	4.02
Glucose + galactose	0.92

Table 3: Effect of different nitrogen source

Nitrogen Source (1%)	Activiy (IU)
Tryptone	3.54
Peptone	0.84
Soyabean meal	0.38
Yeast extract	1.65
Yeast extract + Tryptone	2.24

Bergey's Manual of Determinative Bacteriology (8th Edition). The isolate studied to Gram- negative, coccobacilli, strictly aerobic and non-motile organism. The isolate has been tentatively identified to belong to the genus *Acinetobacter*.

Growth vs. Enzyme activity

The amount of alpha-galactosidase produced was observed after every 2 hr till 24 hr. Maximum activity was observed at 12 hr of growth *i. e.,* during the end of log phase as shown in figure (1). After that alpha-galactosidase yield got reduced due to the consumption of nutrients.

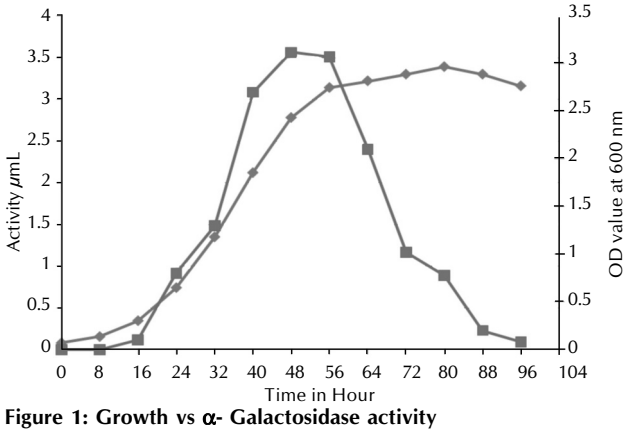

Figure 1: Growth vs α- Galactosidase activity

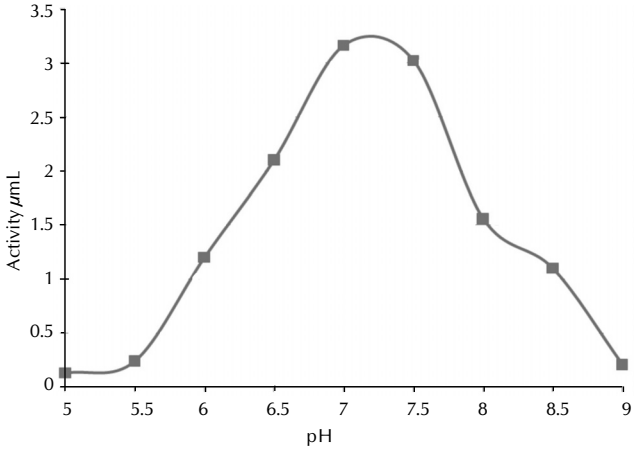

Figure 2: Effect of pH on α- Galactosidase activity

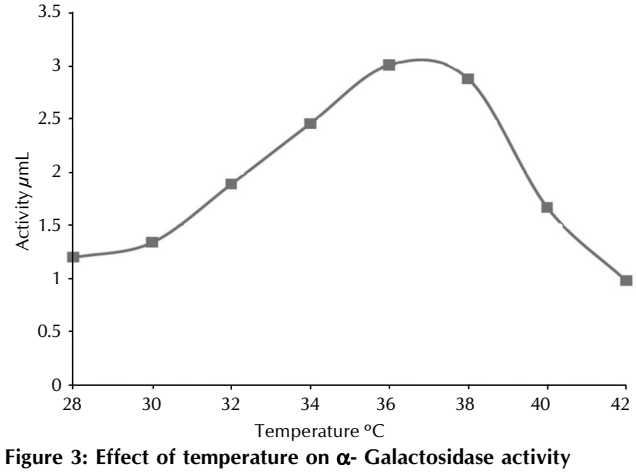

Figure 3: Effect of temperature on α- Galactosidase activity

Effect of carbon, nitrogen, pH and temperature on the enzyme activity

Optimum media conditions for the maximum alpha-galactosidase activity were investigated by studying the effect of carbon source, nitrogen source, pH and temperature. From Figs. 2 and 3, activity was observed at wide pH range and temperature. However, maximum activity was reported at pH 7 and temperature 36ºC. Maximum activity was recorded for both sucrose and raffinose as carbon sources and tryptone as the nitrogen source at the final concentration of 1% (w/v). (Tables 2 and 3)

CONCLUSION

Results present in the Tables (2, 3) and figures (2, 3) indicate that the various compositions in growth media influenced the enzyme production by the bacteria. It appears substrate specificity plays an important role in inducing the enzyme production. Raffinose and Sucrose are the inducers for alpha-galactosidase production. In future studies, intracellular α-galactosidase from *Acinetobacter* will be purified and characterised to determine its suitability as an industrial enzyme in different industries like food, medical and sugar industries for degradation of raffinose oligosaccharides.

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