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RESEARCH ARTICLE

PRODUCTION, OPTIMIZATION AND MOLECULAR CHARACTERIZATION OF GLUCOSE ISOMERASE BY *Enterobacter agglomerans* ISOLATED FROM SOIL

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ABSTRACT

A bacterial strain that produces glucose isomerase was isolated from garden soil and identified as *Enterobacter agglomerans*. Enzyme activity was screened by the formation of cherry red colour and the chemical assay was determined by glucose oxidase enzyme using fructose as substrate followed by the measurement of the amount of glucose. The highest level of extracellular glucose isomerase obtain (41U/ml), temperature around 37°C, pH.6, xylose as a carbon source, peptone as a nitrogen source and incubation time for 36 hours for its higher enzyme productivity. The SDS-PAGE analyses of crude enzyme shows multiple bands, more over along with glucose isomerase some other protein can be produced by the organism.

INTRODUCTION

Glucose isomerase (Xylose isomerase, EC.5.3.1.5) catalyzes the reversible isomerization of D-glucose and D-xylose to D-fructose and D-xylulose, respectively. In the glycolysis pathway phosphoglucose isomerase converts glucose-6-phosphate to fructose-6-phosphate (Snehalata *et al.,*1996). Naturally occurring many organisms has the ability to produce glucose isomerase enzyme. Recent studies have attributed the action of GI to a hybrid shift mechanism. The molecular weight of the glucose isomerase is dependent upon the source of organisms. For example the glucose isomerase gene (xylA) from Knowledge of active-site configuration is required for studying the structure-function relationship of the enzyme. Different approaches have been used to study the active site of GI and to delineate its mechanism of action. These include chemical modification, X-ray crystallography, isotopic exchange (Snehalata *et al.,*1996). The *Streptomyces* sp. SK strain encodes a 388-amino-acid protein (43kDa) (Gil-Jae Joo *et al*., 2005). The ability of enzyme to isomerize a wide variety of substrates such as pentoses, hexoses,sugar alcohols, and sugar phosphates. Although the substrate specificity of the enzyme from different sources changes, the enzyme was able to utilize D-ribose, L-arabinose, L-rhamnose, D-allose, and 2-deoxyglucose as well as most common substrates are D- glucose and D-xylose (Gerald *et al*., 1971).

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The economic feasibility of biomass utilization depends on the hydrolysis of cellulose and hemi-cellulose to glucose and xylose and their subsequent fermentation to ethanol by yeasts. Glucose isomerase is used exclusively in the conversion of starch to sugar. Since fructose is sweeter than glucose, the "sweetness" of the syrup glucose-fructose mixture achieves almost the same sweetness as sugar. The enzyme has the largest market in the food industry because of its application in the production of high-fructose corn syrup (HFCS), an equilibrium mixture of glucose and fructose. Keeping all these in view, a bacterial strain that produces glucose isomerase was isolated from garden soil and identified as *Enterobacter agglomerans*. The present study deals with the cultural condition for glucose isomerase production by *Enterobacter agglomerans* and the protein profile was analyzed in SDS-PAGE.

MATERIAL AND METHODS

Sample Collection and Isolation of Bacteria

The soil was collected from garden in a sterile container and it was brought to the laboratory for further processing. The collected sample was serially diluted up to 10^{-7} dilution using sterile saline as a blank and the diluted samples were plated in to the sterile nutrient agar plates using spread plate method. The plates were further purified by streak plate method using sterile nutrient agar medium. The pure cultures were

inoculated in to sterile nutrient agar slants and nutrient broth for further use.

Screening for Glucose isomerase Producing Organism

The isolated pure cultures were inoculated into a 250 ml conical flask containing 50 ml of culture medium (Peptone 1g, Yeast extract 0.5g, K_2HPO_4 0.3g, $MgSo_4.7H_20$- 0.1g, Xylose 1g,Distilled water 100 ml, pH 7.0) followed by incubating at 28ºC in a shaker waterbath (200rpm) for 24 hours (Chou *et al*.,1976). The supernatant were screened for the production of extracellular glucose isomerase using seliwanoff's reaction. The observation was made to see the Substrate utilized and formation of cherry red colour indicates the presence of fructose. Only positive and better colour forming strain was taken for further study.

Identification of Organisms

The better positive strain that produces maximum Glucose isomerase enzyme was selected and was given for identification in IBMS, University of Madras, Taramani.

Enzyme Production

The enzyme production was carried out by shake flask fermentation using production medium which comprising of xylose as carbon source and amended with peptone and yeast extract as nitrogen sources with pH 7 (Lobanok *et al*., 1998). 500 ml of sterile production broth was prepared in one-liter conical flask and 5% inoculum was transferred aseptically in to the production medium (Peptone 1g, Yeast extract 0.5g, K_2HPO_4 0.3g, $MgSo_4.7H_20$- 0.1g, Xylose 1g, pH 7.0 per 100 ml). The inoculated medium was incubated at 37˚C for 48 hours. The medium was agitated at 200 rpm for better aeration and growth of the organism. The enzyme was extracted for 2 h at 37°C with shaking. The suspension was centrifuged at 10,000 rpm for 10 min. The supernatant was used as the enzyme extract.

Glucose isomerase Enzyme Assay

The glucose isomerase activity was determined indirectly by using the fructose as substrate, followed by the measurement of the amount of glucose produced (Deshmukh *et al*., 1994). The activity was assayed in a reaction mixture containing the enzyme (200µl of an appropriate diluted purified or crude extract) with 10mM $MgCl_2$, 1mM $CoCl_2$ and 15% fructose, in a volume of 1ml. In standard condition, assays were incubated for 30 min at 80 ˚C and the reaction was stopped by cooling the tubes on ice. The amount of generated was determined by glucose-oxidase (GOP-PAP) enzyme system and A540 was measured after 37 ˚C at 15 minutes. One unit of enzyme activity is defined as the amount of enzyme required to liberate 1µmol of product per min under the assay condition.

Estimation of Total Protein

The chemical assay for the total protein content from the sample was determined using Bradford method (Bradford, 1976).

Parameter- Optimization Studies

Effect of incubation time

5ml of inoculum was added in 500ml of sterile production medium and incubated at 37˚C on shaking incubator (150 rpm). 20 ml of incubated culture was aseptically collected 6 hours intervals up to 72 hours. The culture filtrate was examined for the total protein content and glucose isomerase activity (Snehalata *et al.,*1996; Deshmukh *et al*., 1994).

Effect of pH and temperature

The effect of pH and temperature on glucose isomerase production was determined by adjusting the pH with a buffer (4, 5, 6, 7, 8 and 9) and the influence of temperature was assessed by incubating the flasks at 28˚C, 32°C, 37 ˚C, 42°C, 47°C and 52°C.

Effect of Carbon Sources

The effect of carbon source on enzyme production by the organism was studied by incorporating different carbon sources (1 %) such as Glucose, Lactose, Xylose, Starch, Mannitol, sucrose, Maltose.

Effect of Nitrogen Sources

The effect of nitrogen source on enzyme production by the organism was studied by incorporating different nitrogen sources (0.5 %) such as Peptone, Beef extract, casein, gelatin, Ammonium chloride, Ammonium sulphate, Ammonium nitrate, Potassium nitrate.

Partial Purification of Glucose Isomerase

Ammonium sulphate is a particularly useful salt for the fractional precipitation of proteins and enzymes by changing ammonium sulphate concentration. Enzyme extraction was made from culture filtrate using 70% w/v of Ammonium sulfate saturation (chen *et al*., 1979). The mixture was then stored in cold room for 24 hours to precipitate all the proteins. Then the precipitation was separated by centrifugation around 10000 rpm for 10 minutes. Then carefully the supernatant was discarded and the remaining precipitation was dissolved with 2 ml of 0.1M sodium phosphate buffer (pH 7.5) and the mixture was subjected to dialysis in a bag of semi permeable membrane usually made of cellulose acetate with pores of between 1-20 nm in diameter placed in the required buffer so that small molecules can pass freely across the membrane while large molecules are retained. The protein profile and the presence of enzyme were confirmed by SDS- PAGE analysis (Dhungel *et al*., 2007).

RESULTS AND DISCUSSION

12 bacterial strains were isolated from garden soil, but later during screening it was found that 5 bacterial strains showed positive results. Among 5 bacterial strains the better zone formed bacterial strain was identified as *Enterobacter agglomerans* (Fig.1) by standard biochemical tests and it was analyzed by Dept. of Microbiology, IBMS, University of Madras, Taramani.

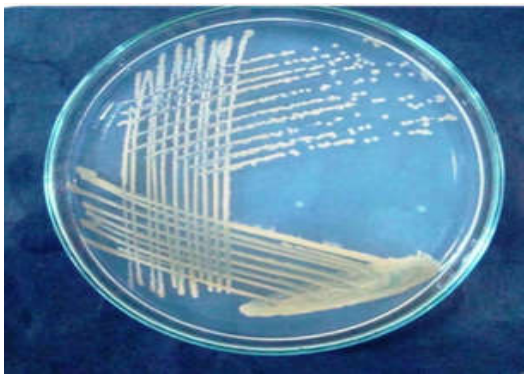


Fig.1. *Enterobacter agglomerans* in nutrient agar medium

Production and assay

In screening the enzyme activity was identified by the formation of cherry red colour (Fig 2) and the chemical assay was determined by glucose oxidase enzyme system using fructose as a substrate followed by the measurement of the amount of glucose.

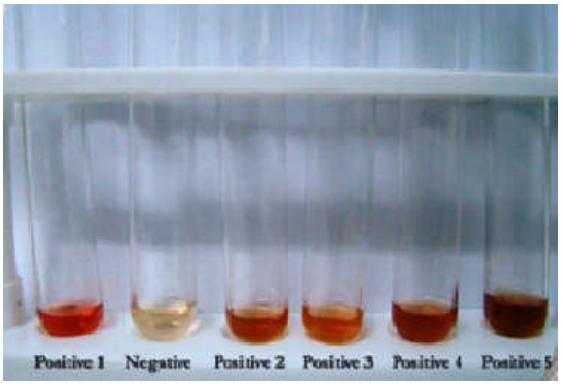


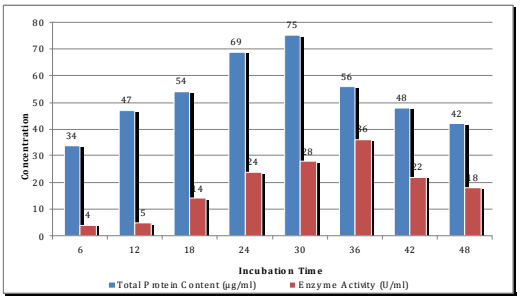
Fig.2. Preliminary screening of glucose isomerase activity

Growth study

The organism has maintained its log phase from 18 hours to around 40 hours. This isolate has shown optimum lag phase and somewhat lengthy log phase. Most of the *Enterobacter* sp. are known to maintained log phase from 16-36 hours (Jyoti Chauthaiwale *et al., 1994*). This variation of log phase timing is based on the nutrient present in the medium and the cultural condition of the organism. The environmental parameters also influence the phase maintaining stationary phase. Even through the extra cellular enzymes are produced from log phase to initial stationary phase, within the phase the production may vary. During growth study, periodical samples were taken and the enzyme activity and total protein content was estimated. The result revealed that the higher production has occurred in the 36 hrs of incubation.

Effect of Time Interval on Enzyme Production

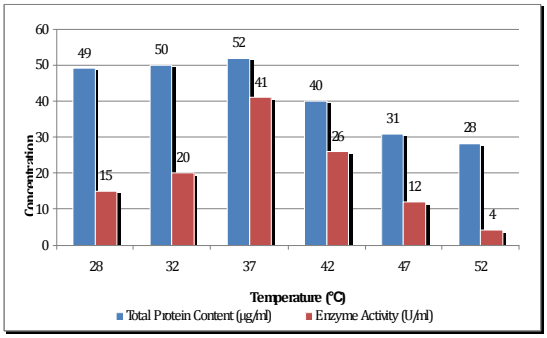
The culture was withdrawn and checked up for enzyme activity with every six hours once for 48 hours. The results revealed that there is gradual increase in production from 24th hours and higher production has occurred at 36th hours (Graph.1). These shows that bacterial isolate should have maintained its log phase from around 24th hour to 36th hour. Besides, it is believed that the higher production of glucose isomerase has occurred in extreme log phase because even though the log phase was maintained between around 24th to 36th hours, the followed drop of production has indicated that the organism should have entered the stationary phase of growth. This variation of log phase timing is based on the nutrient present in the medium and the cultural condition of the organism.



Graph 1. Effect of incubation on total protein and Enzyme Production

Effect of Temperature

From our investigation on effect of temperature on Glucose isomerase production it was found that the mesophilic organisms have potential to produce higher amount of Glucose isomerase (41U/ml) at 37˚C (Graph 2). These indicate that the optimum temperatures for better production of bacterial isolates are 37˚C. Glucose isomerase from *Thermanobacter* sp. displayed apparent temperature optima for activity at between 75˚C and 80 ˚C, and at 65˚C, respectively (Chanyong Lee *et al.,* 1990).

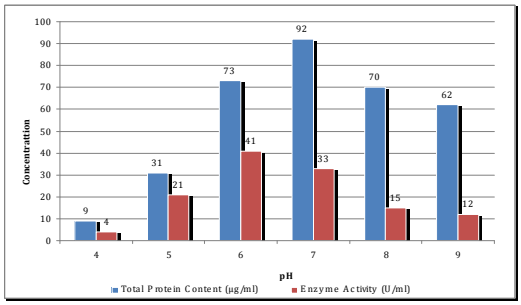


Graph 2. Effect of temperature on total protein and Enzyme Production

Effect of pH

The optimum pH for glucose isomerase activity was determined to be around pH 6.0 when compared to pH 7.0 (Graph 3). This optimum pH is a common feature among all glucose isomerase producing organisms. But the pH requirement of the organism varies from genus to genus. Most GI-producing fermentations are carried out between pH 7.0 and 8.0 without control of pH. *Streptomyces* sp., *Arthrobacter* sp., and *Actinoplanesmissouriensis* are grown at around 38˚C (Anheuser-Busch Inc.,1974). The optimum pH is the ranges between pH 7.0 to 9.0 (Lee *et al*., 1991). The optimum pH of the glucose isomerase is slightly acidic, pH 6.9. It was

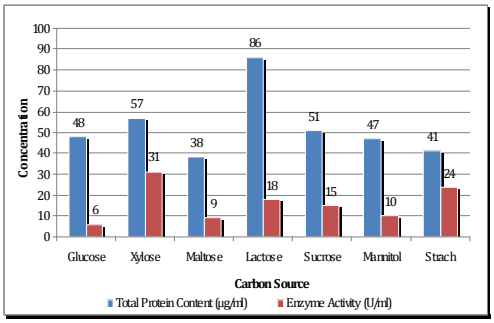
apparently lower than that of enzymes from other *Streptomyes* species (Buckeet al.,1997).



Graph 3. Effect of pH on total protein and Enzyme Production

Effect of Carbon Source

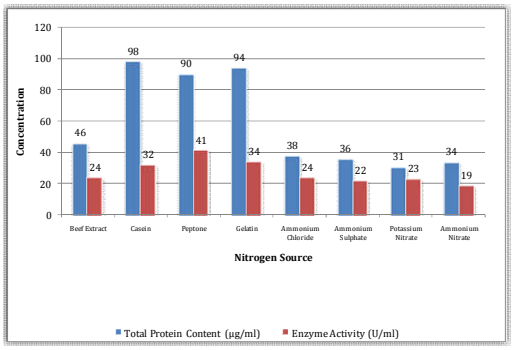
The addition of carbon source in the form of either monosaccharides or polysaccharides may influence the production of enzymes. The isolated strain shows high production around 31 U/ml of production in xylose-amended medium (Graph 4). There are several supports supporting that the *streptomyces* species produces high quantity of glucose isomerase (Chou *et al*., 1976) in xylose amended production medium. The enzyme also called as Xylose isomerase (XI) as it converts xylose to xylulose besides converting glucose to fructose. Hence, xylose was used as the inducer of the enzyme in the culture medium. The enzyme was, then, optimized using glucose as a substrate (Dhungel *et al.*, 2007).



Graph 3. Effect of carbon source on total protein and Enzyme Production

Effect of Nitrogen Source

The nitrogen sources are of secondary energy sources for the organisms, which play an important role in the growth of the organism and the production of enzymes. The nature of the compound and the concentration which we are using may stimulate or down-regulate the production of enzymes. The isolated *Enterobacter* sp. has produced high rate of enzyme in the medium amended with peptone and comparable amount of enzyme has been produced with the medium amended with Yeast extract and Beef extract (Graph 5). In contrast, a negligible amount has been produced with urea, potassium nitrate and ammonium nitrate. Even though, beef extract is an alternate of yeast extract for most of the bacterial growth, as for as GI production is concerned, they are showing different effect (Yoti Chauthaiwale *et al.,* 1994) Peptone, yeast extract, or ammonium salts can be used for GI production, but urea and nitrate are not suitable (Yoshimura *et al., 1966*).



Graph 5. Effect of nitrogen source on total protein and Enzyme Production

SDS-PAGE Analysis

Finally, the protein profile was analyzed in SDS-PAGE; it showed the presence of multiple bands. Obviously, because the medium contain protein source, so unutilized protein also may be present in the exhausted medium. Moreover, along with glucose isomerase some other proteins can be produced by the organisms. But the Presence of protein band nearing the molecular weight 60 Kda confirms the presence of enzyme. It is correlated with the work of Chen *et al.,* (1979). Gil-Jae Joo *et al.* (2005) have reported the *Streptomyces* sp. SK strain producing GI that encodes a 388-amino-acid protein (43kDa).

M-marker, S- Sample

CONCLUSION

The Glucose isomerase enzyme producing bacterial strain was isolated from Garden soil and identified as *Enterobacter agglomerans*. In the production optimization studies, the bacterial strain needs temperature around 37˚C, pH.6, xylose as a carbon source, peptone as a nitrogen source and incubation time 36 hours for its higher enzyme productivity. The protein profile of the crude enzyme was analyzed in SDS-PAGE; it showed the presence of multiple bands. The present study clearly indicates that the Glucose isomerase enzyme are significant importance in the food industry especially production of High Fructose Corn Syrub (HFCS) and ethanol.

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