Isolation, Purification & Characterization of Glucose Isomerase Enzyme form Streptomyces species isolated from Parbhani Region

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Abstract- Bacteria are the rich sources of various enzymes. Streptomyces spices are mostly used for production of various enzymes, antibiotics etc. There product may be extracellular or intracellular. The Glucose Isomerase (GI) is the third most commercially important enzyme. We have carried out screening and isolation of glucose isomerase (GI) producing bacteria from corn field of the Parbhani region. Different isolates was found to be grown on salt starch agar medium at 35 ℃ of which only sreptomyces like colony. Characteristics showing colony was taken and found to be producer of intracellular glucose isomerase on synthetic medium at pH 6.5 containing xylose and along with salt solutions. The optimum conditions for production were 1.5% initial xylose concentration, 1% peptone, 2% Inoculum, pH 6.5 and temperature 35℃ at shaken culture condition. The glucose isomerase was isolated by cell disruption, centrifugation and then it was purified by ammonium sulphate precipitation. The enzyme activity was assayed. The results shown that sreptomyces species was good producers of glucose isomerase.

Introduction

The origin of today's successful development of fructose syrup products lies in the discovery of glucose-isomerizing enzymes. Historically, four different types of enzymes have been termed glucose isomerase. The discovery by Marshall and Kooi in 1957 of the glucose-isomerizing capacity of the enzyme from *pseudomonas hydrophila* was the starting point of the exploitation of this enzyme for the manufacture of HFCS as a substitute for cane sugar. D-Glucose/ xylose isomerase (D-xylose ketol isomerase; EC 5.3.1.5), commonly called to as glucose isomerase (GI), it is the third highest value enzymes after amylase and protease being the other two. According to Wiseman, GI may be the most important of all industrial enzymes of the future. It catalyzes the reversible isomerization of D-glucose and D-xylose to D-fructose and D-xylulose, respectively interconversion of xylose to xylulose serves a nutritional requirement in saprophytic bacteria that thrive on decaying plant material and also aids in the bioconversion of hemicelluloses to ethanol. Isomerization of glucose to fructose is of commercial importance in the production of High-Fructose Corn Syrup (HFCS). Sucrose derived from sugar beet (40%) and sugarcane (60%) was the main sweetener in the world until 1976. The production of HFCS by using glucose isomerase was developed first in Japan and later in the United States. GI gained commercial importance in the United States because of the lack of supply of sucrose after the Cuban revolution in 1958, and it continues to be one of the most important industrial enzymes to this day. The affinity of this enzyme is 160 times lower for glucose than for xylose, it is sufficient for the enzyme to be commercially significant. Production of the enzyme required xylose in the growth medium and is enhanced in the presence of arsenate. Later, a xylose isomerase activity, which was independent of xylose, was found in *Escherichia intermedia*. A similar glucose Isomerase activity, which catalyzed the isomerization of both glucose and mannose to fructose, was isolated from paracolobacterium *aerogenoides*. The glucose isomerases produced by heterolactic acid bacteria require xylose as an inducer and are relatively unstable at higher temperatures. Of these

glucoseisomerizing activities, xylose isomerase (EC 5.3.1.5) is the most suitable for commercial applications. It is heat stable and does not require expensive cofactors such as NAD1 or ATP for activity. The potential for using sugar substitutes produced from starch was proposed by several workers. Glucose Isomerase convert glucose to fructose offers several advantages, such as (i) specificity of the reaction, (ii) requirement of ambient conditions of pH and temperature, and (iii) no formation of side products. Therefore, enzymatic conversion is preferred to chemical isomerization of glucose to fructose, and today the process involving GI has undergone considerable expansion in the industrial market. Bacteria and actinomycetes were found to produce GI that is active in the absence of arsenate. Among the heterolactic acid bacteria, Lactobacillus brevis produced the highest yield of enzyme. The enzyme was active at low pH but unstable at high temperature and hence was not suitable for economic exploitation. Reports on extracellular secretion of GI are not common. Extracellular GI has been reported to be produced by Streptomyces glaucescens and S. flavogriseus, for which the release of the enzyme from the cells was attributed to a change in the cell wall permeability and partial lysis of the cells. The extracellular xylose isomerases from chainia sp. and an alkalothermophilic Bacillus sp. have been purified to homogeneity by conventional purification techniques such as gel filteration, ion exchange chromatography and preparative polyacrylamide gel electrophoresis. As well as streptomyces sp., several Bacillus species are good producers of GI. The occurrence of GI in a few yeasts such as candida utilis and candida boidinii has been documented. Aspergillus oryzae is the only fungus which is reported to possess GI activity. The existence of GI in barley malt and wheat germ has been reported. The organisms that are commercially important as GI producers are listed in since GI is a subject of great commercial importance, much of the information on new producer organisms and on developed processes is in the form of patents. The knowledge of specific properties of the enzyme, such as its stability, substrate specificity, and metal ion requirement, is important to prevent its inactivation and to assess its suitability for application in HFCS production.

Material & Methods

Isolation of Streptomyces Specie from corn field of Parbhani Region

Collected soil sample from corn field was inoculated on solid & liquid salt starch agar medium after serial dilution. Confirmation of sample was done with Gram Staining and other biochemical tests like Oxidase Test, Citrate Utilization Test, Starch Hydrolysis Test, Casein Hydrolysis Test, Sucrose Test, Gelatin Hydrolysis, Lipid Hydrolysis Test and Hydrogen Sulfide Test.

Production Process of Glucose Isomerase Enzyme

Production media contain Xylose (0.75%), Peptone (1.00%), Yeast Extract (0.5%) and $MgSo_4.7H_2O$ (0.1%) as the components, P^H of media should be maintained upto 7.0 even after autoclaving. After sterilization production media (100 ml.) is inoculated with pure culture. We will provide overnight incubation. After overnight incubation 2.00 ml. of inoculated production media will be transferred to new fresh flask of 100 ml. of production media. We will repeat above process again and whole inoculated production media will be transferred to 1000 ml. of production media and it will be incubated for 24 Hr. Production of enzyme will take place with in this incubation time.

Extraction and Purification of Intracellular Enzyme

Cells of 24 hr incubated production media were harvested and washed with distilled water, cells were suspended in distilled water and treated with homogenizer for 10 to 20 minute. Homogenized cells were centrifuged at 20,000g for 20 min minute, removed whole cells and debris; the supernatant was brought to 30% saturation of ammonium

sulphate. The precipitate was removed by centrifugation at 20,000g for 15 min and the supernatant was brought to 70% saturation of ammonium sulphate. Pellet collected by centrifugation at 20,000g for 30 min was dissolved in distilled water. Purification of enzyme was done by Salting out method.

Characterization of Various Parameters of Purified Enzyme

1. Enzyme Assay
2. Isomerase Activity
3. Protein Content
4. Fructose Content
5. Effect of Temperature

All the above mentioned parameters are verified by standard protocols.

Results

Isolation and Screening for Bacteria

After completion of the incubation pale yellow colonies were observed on salt starch agar plates.

Grams Staining

After performing staining procedure slides were observed under microscope. The following characters were observed; Gram positive, violet color, filamentous rod shaped bacteria were examined under microscope.

Biochemical Tests:

Sr.No.	Tests	Results
1	Starch Hydrolysis	Positive
2	Casein Hydrolysis	Positive
3	Sucrose Test	Positive
4	Catalase Test	Positive
5	Oxidase Test	Positive
6	H_2S Production	Positive
7	Citrate Utilization	Positive
8	Lipid Activity	Positive
Result		***Streptomyces***

Production and Extraction of GI

After the incubation of production media the enzyme was produced intracellular and the enzyme was extracted by the centrifugation.

Purification of GI

The crude enzyme was purified by the ammonium sulphate precipitation i.e. salting out method.

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Estimation of Standard Protein by Lowry's Method
The amount of enzyme produced i.e. unknown concentration of crude enzyme was found to be 40.00 mg/ml.

Estimation of Fructose by Seliwanoff's Test
The amount of fructose produced i.e. unknown concentration of fructose was found to be 700 mg/ml.

Effect of pH on Enzyme Activity
The crude enzyme shows its activity for conservation of glucose in the alkaline region with optimum pH 7.0-8.0. The effects of pH on enzyme activity between pH 5.5-10 were measured by spectrophotometer, the effect of pH on enzyme activity.

Effect of Temperature on Enzyme Activity
The crude enzyme shows its activity at optimum temperature was found to be at 80^{c}.

Discussion
Our isolated strain was found to be the producer of GI. Next step was to design of medium for GI production; this was done by standard references of W.P. CHEN. The next work was on inoculums preparation, after that the production was started and completed in two days, the completion of production resulted into extraction of enzyme which was carried out by firstly separating biomass because our interest of enzyme was intracellular. This was done by spinning the fermentation medium at 12,000g and then homogenized to lyses the cells and enzyme was separated and purified by ammonium sulphate precipitation and then on dialysis. The extracted enzyme were checked for protein contents by Lowry method and the concentration of unknown protein was found to be assayed for enzyme activity for the different parameters such as temperature and pH and the isolated enzyme found to be optimum at 80 °C and pH 7. Isomerase activity of enzyme was checked by the seliwanoff's test and the amount of fructose produced was found to be 700 mg/ml. From this work we had ultimately concluded that the our regions Streptomyces can also produced significant amount of glucose isomerase that can decrease cost of purchasing the other strains and also our strain can limit the temperature variation because of our climatic condition there is a no need to maintain the low and high temperature.

References
[1] Anonymous (1973) Beckman glucose analyzer operation manual. Beckman Instruments Inc., Fullerton, Calif.
[2] Anonymous (1973) Corn syrup from immobilized enzyme. Chem. Eng. News 51:36.
[3] Bidur Dhungel, Manoj Subedi 1, Kiran Babu Tiwari, Upendra Thapa Shrestha, subarna Pokhrel and Vishwanath Prasad Agrawal 1 Thermostable glucose isomerase from psychrotolerant streptomyces species Universal science College, Pokhara University, Kathmandu, Nepal Department of Enzyme Engineering, Seoul National University, Korea 17.6.07
[4] Bucke, C. (1977) Industrial glucose isomerase, p. 147-171 In A. Wiseman (ed.), Topics in enzyme and fermentation biotechnology. Halsted Press, New York.
[5] Chou C.C., Ladisch M.R. and Tsao G.T. Studies on Glucose Isomerase from a streptomyces species school of Chemical Engineering, Purdue University, West Lafayette, Indiana 47907,1976.

[6] Danno, G. (1970) Studies on D-glucose-isomerizing enzyme from Bacillus coagulans, strain NH-68. Part V. comparative study on the three activities of D-glucose, D-xylose, and D-ribose isomerization of the crystalline enzyme. Agri. Bio. Chem. 34:1805-1814.

[7] Giovenco, Morisi, and Pansolli. (1973) Properties of free and immobilized glucose isomerase. FEBS Lett. 36:57-60.Hagedorn,C. 1976. Influences of soil acidity on streptomyces populations inhabiting forest soils. Appl. Environ. Microbial.32:358-375.

[8] Ibrahim H. Abbas Glucose isomerase from streptomyces rubiginosus- potential molecular weight standard for small-angle X-ray scattering A Biological and Biochemical studies of Actinomycetes Isolated from Kuwait Saline soil-Kuwait. Department of Botany, Faculty of science-Benha University. Egypt.

[9] Jenson, H.L. (1928) Actinomycetes acidophilus n. sp. a group of acidophilus actinomycetes isolated from the soil. Soil Sci. 25:225-233.

[10] Joseph,R.,M.S. Shanthamma, and V.S. Murthy. (1977) Isolation of streptomyces having high glucose isomerase activity and assessment of their efficiency in the production of fructose syrups. J. Food Sci. Technol. 14:73-77.

[11] Khan, M.R., and S.T. Williams. (1975) Studies on the ecology of actinomycetes in soil.VII. Distribution and characteristics of acidophilic actinomycetes. Soil Biol. Biochem. 7:345-348.

[12] Koen Dekker, Hideo Yamagata, Kenji Sakaguchi, and Shigezo Udaka xylose (Glucose) Isomerase Gene from the Thermophilic Thermus Thermophilus: cloning, sequencing, and comparison with other Thermostable xylose Isomerases Faculty of Agriculture, Nagoya University. Chikusa-ku, Nagoya 464,1 and Nihon Shokuhin Kako Co. Ltd., Marunouchi. Chiyoda-ku, Tokyo 100,2 Japan Received 27 November 1990/Accepted 5 March 1991.

[13] Pinar Calik Vahideh Angardi Nazife Isik Haykir and Ismail Hakki BYAC (2008) Molecular and industrial aspects of glucose isomerase. Glucose isomerase production on a xylan-based medium by Bacillus thermoantarcticus Department of Chemical Engineering, Industrial Biotechnology and Metabolic Engineering Laboratory, Middle East Technical University, 06531 Ankara, Turkey Department of Food Engineering, Hacettepe University, 06532 Ankara, Turkey Received.

[14] Snehalata H. Bhosale, Mala B. Rao and Vasanti V. Deshpande (2005) Molecular and Industrial Aspects of Glucose Isomerase Division Of Biochemical Sciences, National Chemical Laboratory, Pune-411008. India Regulation of xylose metabolism in recombinant saccharomyces cerevisiae Laura Salusjarvi 1. Matti Kankainen2, Rabah Soliymani, Juha-pekka Pitkanen1, Merja Penttila1 and Laura Ruohonen1 J. Appl. Cryst. 38, 555-558.

[15] Song H. Bok, Martin Seidman and Paula W. Wopat (1984) Selective Isolation of Acidophilic streptomyces strains for Glucose Isomerase production Hoffmann-La roche Inc., Nutley, New Jersey 07110;1 A.E. Staley Manufacturing Co. Decatur, Illinois 625252; and Genetics Institute, Boston, Massachusetts 021153 Received.

[16] Chen W.P., Anderson A.W. and Han Y.W. . Production of Glucose Isomerase by Streptomyces flavogriseus Department of Microbiology, Oregon State University, Corvallis, Oregon 97331 Department of Science and Education Administration-Federal Research, U.S. Department of Agriculture, Oregon State University, Corvallis, Oregon 97331 Technical paper no. 4893, Agricultural Experiment Station, Oregon State University.

Table 1- Estimation of Standard Protein by Folin Lowry's Method

Sr. No.	Conc. of Standard proteinMg/ml	Volume of stock (ml)	D/W (ml)	Solution D (ml)	I	F.C.R. (ml)	I	O.D. At750nm
1	0.5	0.2	1.8	5	N	0.5	N	0.10
2	1.0	0.4	1.6	5	C	0.5	C	0.25
3	1.5	0.6	1.4	5	U	0.5	U	0.33
4	2.0	0.8	1.2	5	B	0.5	B	0.42
5	2.5	1.0	1.0	5	A	0.5	A	0.50
6	3.0	1.2	0.8	5	T	0.5	T	0.62
7	3.5	1.4	0.6	5	I	0.5	I	0.75
8	4.0	1.6	0.4	5	O	0.5	O	0.83
9	4.5	1.8	0.2	5	N	0.5	N	0.89
10	5	2.0	0.0	5	AT	0.5	AT	0.95
11	Blank	0.0	2.0	5	RT	0.5	RT	0.00
12	Sample	2.0	0.0	5	10Min	0.5	30Min	0.60

Table 2- Estimation of Fructose by Seliwanoff's Test

Sr. No.	Conc. Of fructose Mg/ml	Volume of fructose (ml)	Volume of D/W (ml)	Volume of Seliwanoff's reagent (ml)	O.D. At 540 nm
1	100	0.2	3.8	5	0.07
2	200	0.4	3.6	5	0.15
3	300	0.6	3.4	5	0.16
4	400	0.8	3.2	5	0.25
5	500	1.0	3.0	5	0.28
6	600	1.2	2.8	5	0.33
7	700	1.4	2.6	5	0.39
8	800	1.6	2.4	5	0.40
9	900	1.8	2.2	5	0.50
10	1000	2.0	2.0	5	0.56
11	Blank	0.0	4.0	5	0.00
12	Unknown	-	-	5	0.39

Table 3- Effect of Temperature on enzyme activity

Sr. No.	Temperature	Specific activity
1	40	0
2	50	1
3	60	1.8
4	70	6
5	80	10
6	90	6.5

Table 4- Effect of pH on Enzyme Activity

Sr. No.	pH	Specific Activity
1	5.5	0.4
2	6	0.5
3	6.5	1.4
4	7	2.5
5	7.5	2
6	8	1.6

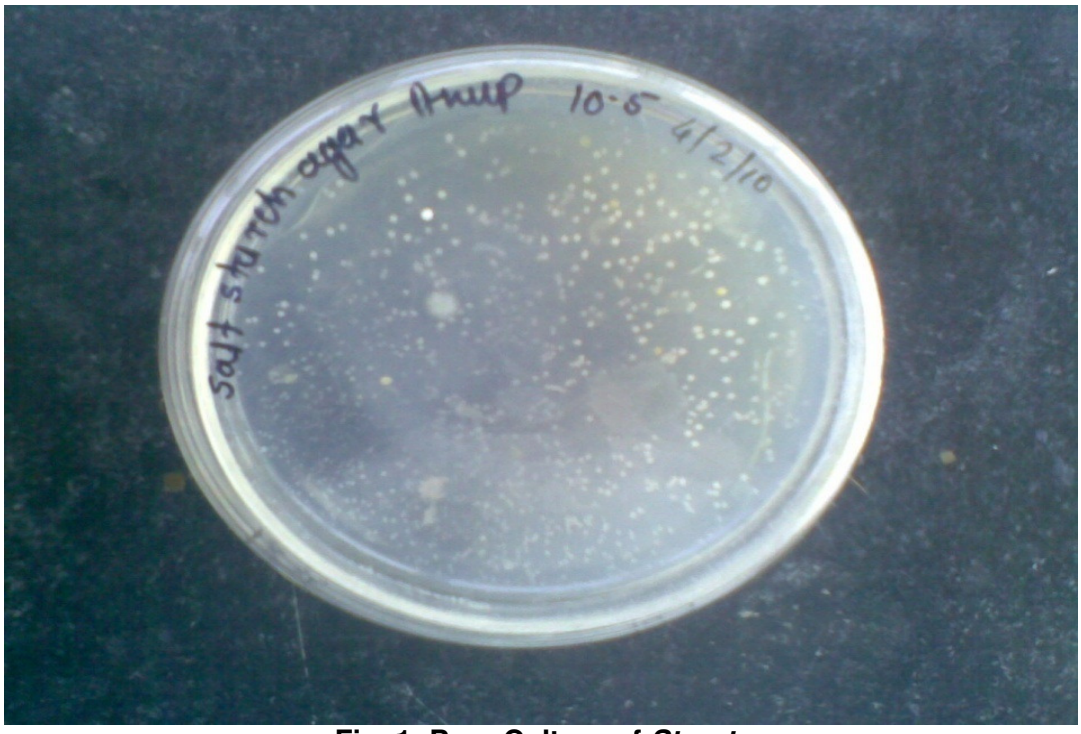

Fig. 1- Pure Culture of *Streptomyces*

Fig. 2- Casein Hydrolysis Test

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Fig. 3- Carbohydrate Fermentation

Fig. 4- H_2s Test

Fig. 5- Amylase Test

Fig. 6- Extraction of GI from Streptomyces

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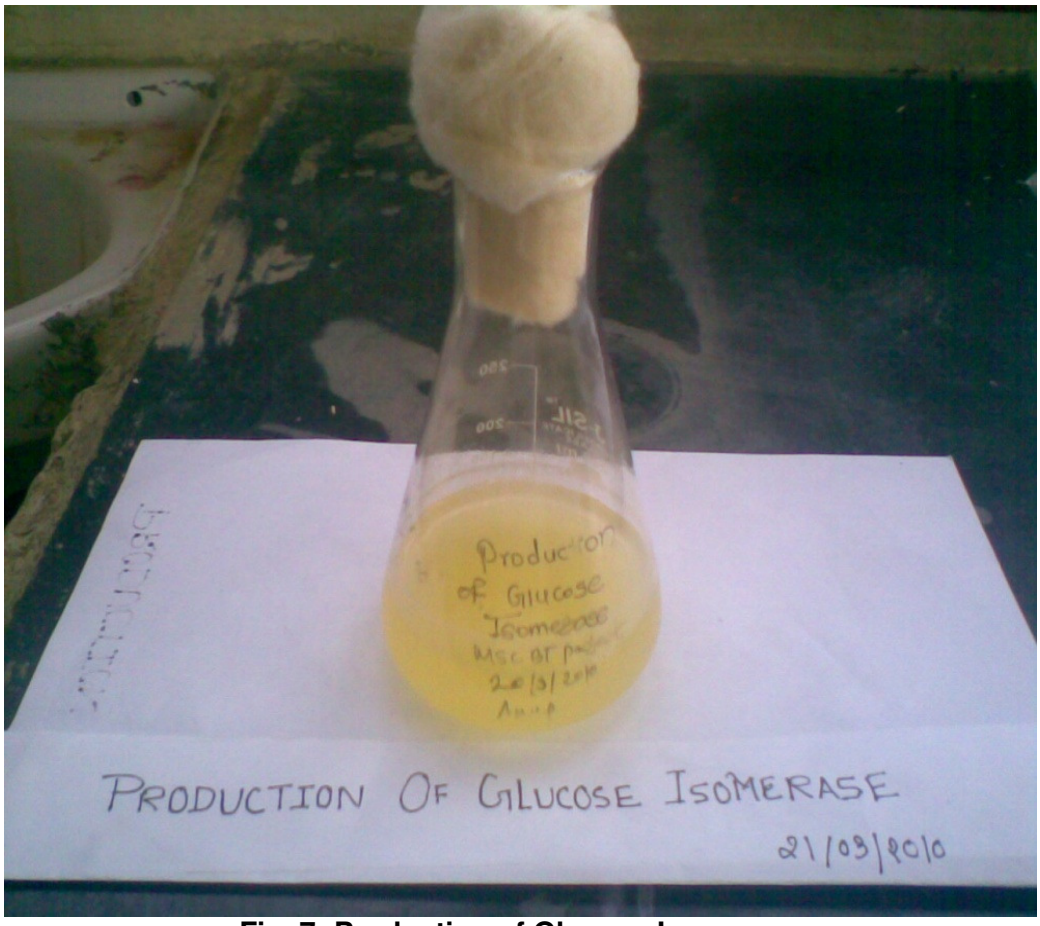

Fig. 7- Production of Glucose Isomerase