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Glucose Isomerase Production and Its Application In Various Field

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ABSTRACT

The activity of Glucose Isomerase was first time observed in 1953 by Mitsuhashi and Lanpen. Marshall and kooi discovered the glucose isomerizing capacity from Pseudomonas hydrophila. Glucose Isomerase activity is naturally observed in many bacterial species such as in Escherichia coli, Lactobacillus species, Flavobacterium species and also in yeasts such as in Saccharomyces cerevisia and also in Candida utilis. These organisms give high yield and are easy to handle and culture. Effect of certain inhibitors, and activators, affecting the catalytic activity of the enzyme are mentioned. Method of production of this enzyme is also described, followed by its extraction and how we do the bioassay. This method mentioned over here is most applicable throughout the industries and research institutes for the production of Glucose Isomerase. It catalyzes the reversible isomerization of D-glucose. It also catalyzes the isomerization of D-xylose to D-fructose. For the production of High fructose corn syrup this conversion is very important. It is used in food additives in food industry, in Beverage industry and also in bread making and in confectionary products. It also has uses in medicinal industry. It is also used in ethanol production. All these applications of Glucose Isomerase make it an important enzyme in industries.

Keywords : Xylose Isomerase, Lactobacillus, Fructose Corn syrup, Food additives.

I. INTRODUCTION

D-Glucose isomerase is also known as Xylose Isomerase. It is one of the most important enzyme known. It is one of the three highest tonnage value enzymes. The other two besides it are Protease and Amylase. In 1953 Mitsuhashi and Lanpen first observed the activity of Glucose isomerase in a bacteria Lactobacillus pentosus **(Bhosale et al., 1996)**. In 1957 Marshall and Kooi discovered the glucose isomerizing capacity of glucose isomerase from Pseudomonas hydrophila **(Bhasin andModi, 2012)**In prokaryotes it is widely distributed. After the discovery of glucose isomerase in Pseudomonas hydrophila, a large number of actinomycetes and bacteria were found which produce this enzyme. They discovered the enzyme that is active in the absence of arsenate**(Chen et al., 1979)**. Lactobacillus brevis produced the enzyme in highest yield among all the heterolactic acid bacteria. At low pH the enzyme was active but at high temperature it is unstable and hence for economic exploitation it was not suitable **(Bhosale, et al., 1996).**

Reports on extracellular secretion of Glucose isomerase are not known so much. Streptomyces glaucescens and S. flavogriseus has been reported to produce extracellular glucose isomerase, for which the secretion of the enzyme from the cells was attributed to a change in the permeability of the cell wall and lysis of the cells partially. The glucose isomerase from Chainia sp. and an alkalo thermophilic Bacillus sp. which produce it extracellularly **(Sayyedet al., 2010).**
In a few yeasts such as Candida utilis and Candida boidinii the occurrence of glucose isomerase has been reported. The only fungus in which glucose isomerase

activity is reported is Aspergillus oryzae. It is also reported that in barley malt and wheat germ glucose isomerase activity is present **(Bhosale et al., 1996)**.

Glucose isomerase catalyzes the equilibrium reversible isomerization of D-glucose. It also catalyzes the isomerization of D-xylose to D-fructose and D-xylulose **(Kamal et al., 2013).** For the production of High Fructose Corn Syrup conversion of glucose to fructose by glucose isomerase is commercially very important **(Jia et al., 2017)**. This process converts glucose into such a mixture which is very sweet like sucrose **(Sayyed et al., 2010)**.

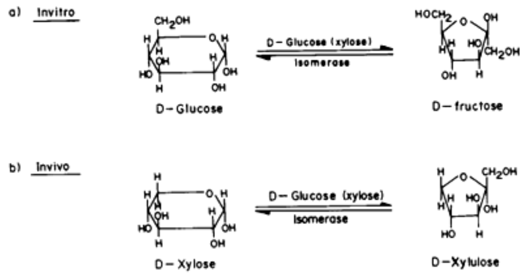

Figure 1. Reactions catalyzed by Glucose Isomerase (a) In vitro reaction (b) In vivo reaction.

(Bhosale et al., 1996)

Sources of Production

In the United States glucose isomerase gained importance commercially because after the Cuban revolution in 1958 there is lack of supply of sucrose. It is one of the commercially important enzyme reported till date **(Wang et al., 2015)**. The affinity of glucose isomerase is lower 160 times for glucose than for xylose. For the production of glucose isomerase xylose is required in the growth medium and its growth is enhanced in the presence of arsenate. A glucose Isomerase activity, was isolated fromparacolobacterium aerogenoideswhich catalyzed the isomerization of both glucose and mannose to fructose **(Srivastava et al.,2010)**.

Table1. Commercial producers of Glucose Isomerase

Organism	**Yield**	**Temp (⁰C)**	**pH**	**References**
Actinoplanes missousriensi	2,500-35,200	75	7.0	**(Anheuser, 1974)**
Bacillus licheniformis	10,500	70	NC*	**(Boguslawski,1982)**
Streptomyces wedmorensis	560-2500	70	7.2	**(duPreez, 1987)**
Streptomyces oilvochromogenes	4,800-11,440	60	7.5	**(Huang, 1985)**

NC, not clarified

Review of Literature:

No. of publications regarding Glucose isomerase, trend as well as areas of research related with the Glucose isomerase.

Table 2. No. of publications along with the year range regarding the enzyme Glucose Isomerase with the trend of research.

Years	No. of Publication	Trend of research
1955-1964	2	Conversion of glucose to fructose by Glucose Isomerase. Enzymatic control of metabolic pathways. Purification and characterization is done
1965-1974	11	Expression of glucose isomerase is done.Purification and characterization is done from Bacillus stearothermophilus. Immobilization of Glucose Isomerase to ion exchange material is done.
1975-1984	69	Activity of Glucose Isomerase in the fermentation of lignocelluloses hydrolysatesis done. X-ray analysis of Glucose Isomerase is done. Structure of D-Glucose Isomerase fromArthrobacter strain B3728 is done. Comparative study of the enzyme is done.
1985-1994	156	Inhibitors of Glucose Isomerase are seen. Saccharification and Isomerization is done. Separation of glucose isomerase from different species is done.
1995-2004	147	Studies on the microbial Glucose Isomerase are done. Role in diseases.
2005-2014	218	Glucose Isomerization. Biofuel production. Effect of Different substrates on its activity is done.
2015-2017	43	Food usage. Diagnostics. Diffraction quality crystals preparation. Work on Thermodynamics and kinetics of Glucose isomerase crystals.

Source: NCBI Database

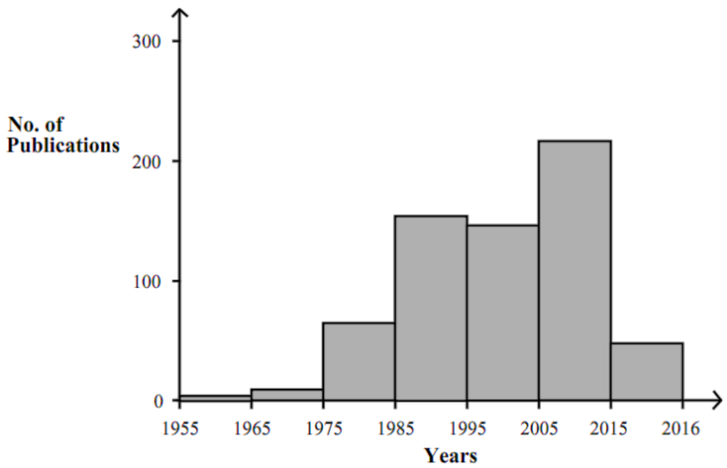

Figure 2. Plot between no. of publications against year range

Source: NCBI Database

Production and extraction:

Glucose Isomerase producing organisms

Table 3. List of all Bacterial Sources producing Glucose isomerase

Actinomyces olivocinereus Actinomyces phaeochromogenes Actinoplanes missouriensis Aerobacter aerogenes Aerobacter cloacae Aerobacter levanicum Bacillus stearothermophilus Bacillus megabacterium Bacillus coagulans Brevibacterium incertum Cortobacteriumhelvolum Escherichia freundii Escherichia intermedia Escherichia coli Flavobacterium arborescens Flavobacterium devorans Lactobacillus brevis Lactobacillus buchneri Lactobacillus fermenti Lactobacillus mannitopoeus Lactobacillus gayonii Lactobacillus fermenti Lactobacillus plantarum Lactobacillus lycopersici Lactobacillus pentosus Leuconostoc mesenteroides Microbispora rosea Microellobosporia flavea Micromonospora coerula Nocardia asteroides Nocardia corallia	Paracolobacterium aerogenoides Pseudomona shydrophila Staphylococcus bibila Staphylococcus flavovirens Staphylococcus echinatus Streptococcus achromogenes Streptococcus phaeochromogenes Streptococcus fracliae Streptococcus roseochromogenes Streptococcus olivaceus Streptococcus californicosStreptococcus venuceus Streptococcus virginial Streptomyces olivochromogenes Streptomyces venezaelie Streptomyces wedmorensis Streptomyces griseolus Streptomyces glaucescens Streptomyces bikiniensis Streptomyces rubiginosus Streptomyces achinatus Streptomyces cinnamonensis Streptomyces fradiae Streptomyces albus Streptomyces griseus Streptomyces hivens Streptomyces matensis Streptomyces nivens Streptomyces platensis Streptosporangium album Streptomyces oulgare Zymononas mobilis

(Bhosale et al., 1996)

Glucose isomerase producing organisms

Table 4. List of all Yeast source producing Glucose isomerase

Organisms	**References**
Candid utilis	**(Harner et al.,2015)**
Saccharomyces cerevisia	**(Mert et al., 2017)**
Pachysolen tannophilus	**(Bhosale et al., 1996)**
Pichia stipitis	**(Bengtsson et al., 2009)**
Candida tropicalis	**(Harner et al.,2015)**
Candida shehatae	**(Harner et al.,2015)**
Schizosaccharomyces pombe	**(Bhosale et al., 1996)**

II. MICROBIAL PRODUCTION AND GROWTH

Isolation of Streptomyces Specie

Sample is collected from the soil. Then serial dilution of sample is done and after that it is inoculated on starch agar medium. It is necessary to confirm the sample and confirmation is done with the help of biochemical tests and by the procedure of Gram staining. After the process of Gram staining slides are observed under microscope. The following characters are observed they are gram positive and the color which is seen is violet. The bacteria are filamentous rod shaped. The colonies are grown on the agar plates. After the process of incubation when colonies are grown on agar medium pale yellow color colonies are seen. The desired colony is picked for further use **(Srivastava et al., 2010).**

Production Process of Glucose Isomerase Enzyme

Production media contain

- ✓ Xylose (0.75%)
- ✓ Peptone (1.00%)
- ✓ Yeast Extract (0.5%)
- ✓ $MgSO_4.7H_2O$ (0.1%)

pH of media should be maintained up to 7.0 **(Srivastava et al., 2010).**

Inoculation

The media after preparation is sterilized and after sterilization it is inoculated with the help of colony which were grown on the plates which we prepared earlier. The media after inoculation is incubated for overnight. After overnight incubation 2.00 ml. of inoculated production media will be transferred to new fresh flask of 100 ml. of production media. 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 within this incubation time **(Nwokoro, 2015).**

Extraction and Purification of Intracellular Enzyme

After the incubation time of 24 hr the cells from the production media were harvested and washing is done with distilled water, cells were suspended in distilled water and treated with homogenizer for 10 to 20 minute. Homogenized cells were centrifuged at 20,000rpm for 20 min minute, removed whole cells and debris. The supernatant is removed;the supernatant was brought to 30% saturation of ammonium sulphate **(Pinar et al., 2008).**

Centrifugation

The precipitate was removed by centrifugation at 20,000rpm for 15 min and the supernatant was brought to 70% saturation of ammonium sulphate. Pellet collected by centrifugation at 20,000rpm for 30 min was dissolved in distilled water. Then Purification of enzyme was done. The supernatant which came can be used for the enzyme bioassay**(Srivastava et al., 2010)**

Glucose isomerase assay

The production of glucose isomerase was detected the following method as described by (**Takasaki, 1966**). Enzyme reaction was performed using following chemical mixture in which following components are present **(Sathya and Ushadevi, 2014):**

- ✓ 0.4ml of 1M D-glucose
- ✓ 1ml of potassium phosphate buffer (pH.7.5)
- ✓ 0.2ml of 0.1M $MgSO_4.7H_2O$
- ✓ 0.2ml of 0.01M $CoCl_2.6H_2O$
- ✓ 0.8ml of enzyme solution
- ✓ After addition of all these substances the final volume was made up to 4 ml in deionized water.
- ✓ The solution is incubated for 30min at 65°C.
- ✓ The reaction was stopped by adding 5ml of resorcinol reagent and then heated at 100°C for 5min.
- ✓ After heating it is then store on ice for 10min.
- ✓ Centrifuged at 10000rpm for 15min. The absorbance and wavelength scan was read at 485nm using UV spectrophotometer with a standard fructose as a reference. 1 unit of the

glucose isomerase activity was apparent as the total of the enzyme that produced 1μmol of D-fructose per min under the assay condition was employed**(Sathya and Ushadevi, 2014).**

Significance of glucose isomerase:

Now a day's glucose isomerase is used widely for the production of many industrially importance things. Some of the important uses of glucose isomerase are discussed below.

Table 5. Industrial applications of Glucose isomerase

INDUSTRY	PRODUCT	APPLICATIONS	REFERENCE
Food industry	• Corn Syrup, HFCS • Food additive	• For Production of corn syrup containing a mixture of glucose and fructose • Used as a sucrose substitute • High fructose syrups extend the shelf life in foods	**(Parker et al., 2010)**
Beverage industry	• Ethanol • Fructose (sweetener)	• Used in Ethanol production • To produce HFCS which help in giving sweet taste to the beverage • Less expensive than other sugar substitute • Used in soda • Used for sports drinks. • Used as sweetener in drinks • Used for isomerization of xylose to xylulose, which can be ultimately fermented to ethanol by conventional yeasts • Used for isomerization and fermentation of xylose to ethanol simultaneously	**(Bhosale et al., 1996)**
Baking industry	• Bread making • Confectionary products	• Used for production of High fructose syrup which provides better browning than	

	• Baking as humectants • Pastries • Biscuits	unprocessed glucose in baked goods • Produce ethanol, helps to ferment better • Help in dough rising	**(Parker et al., 2010)**
Canning industry	• Flavor Enhancers • Preservatives • Canned items such as canned fruit, sauce, jelly, ketchup, jams, pickles	• Used in production of HFCS which is used as a source of artificial sweetener • Artificial flavor • Flavor enhancer • Increases shelf life of canned food, drink.	**(Kamal et al., 2013)**
Confectionery industry	• Dough making • Softness and crystallization control (Bread, Buns, cakes) • Dextrose syrup in Ice cream production • Browning of baked items	• Used to produce a softer texture in these items • High fructose syrup provides better browning than unprocessed glucose in baked goods	**(Bhosale et al., 1996)**
Medicinal use	• Medicines of diabetic, cardiovascular, patients • Low caloric sugar such as Sucral sweetex for obese people and those having metabolic syndrome	• A diabetic sweetener • Not influence the glucose level in blood • Slowly reabsorbed by the stomach • Used in diabetic medicines • Does not affect level of insulin hormone.	**(Parker et al., 2010)**
Energy	• Biogas &Ethanol	• Production of Biofuels by conversion of xylose to ethanol (xylose isomerase).	**(Volkin and Klibanov, 1989)**

Production of high fructose corn syrup

High fructose corn syrup is a sweetener. It is produced by the enzymatic activity of glucose isomerase; it acts on the corn starch converts some of its glucose into fructose. In the early 1970's high fructose corn syrup is first marketed **(White et al., 2016).** It is now considered as a substituent for sucrose in soft drink production. In the United States corn starch manufactured by wet milling process is considered as the important raw material for the production of high fructose corn syrup. Three major processes are involved for the production of high fructose corn syrup

- ✓ Liquefaction of starch by □-amylase
- ✓ Saccharification of starch by the combined action of amyloglucosidase and a debranching enzyme
- ✓ Isomerization of glucose by GI**(Visuri and Klibanov, 1987)**

In the result of all these processes the final product which came is the mixture of fructose and glucose and it has high sweetening capacity as compared to that of sucrose. In other parts of world rather than corn starch other things like rice and wheat is also used for its production in minor extents **(Bhosale et al., 1996).** In high fructose corn syrup water is present as 24% and rest of the amount includes glucose or fructose and 0.5% of glucose oligomers are also present which is in the unprocessed form **(Visuri and Klibanov, 1987)**. As compared to granular sucrose this syrup is easier to handle. In other nations Soft drink makers such as Coca-Cola and Pepsi use sugar, but in 1984 switched to HFCS in the U.S **(Zargaraan et al., 2016).** As a diabetic sweetener D-fructose plays an important role because it is only slowly reabsorbed by the stomach and does not influence the glucose level in blood. The major uses of HFCS are in the, canning, beverage, baking and confectionery industries. As in the result of its uses the causes of obesity occur in the people so its use starts to decline (**Snehalata et al., 2005).**

Ethanol production

The isomerization of both glucose and xylose is catalyzed by Glucose isomerase. In the isomerization of xylose to xylulose this property of the enzyme is used, which can be ultimately fermented to ethanol by conventional yeasts **(Chanitnun and Pinphanichakarn, 2012).** In view of the rapid depletion of fossil fuels bioconversion of renewable biomass to fermentable sugars and ethanol is important.

The economic feasibility of biomass utilization depends on the hydrolysis of cellulose and hemicellulose to glucose and xylose and their subsequent fermentation to ethanol by yeasts **(Ko et al., 2016)**. On the bioconversion of cellulose until recently, the research efforts were focused **(Bhosale et al., 1996)**. The bioconversion of lignocelluloses and agricultural wastes efficiency relied mainly on the effective utilization of the hemicellulose component of biomass shifted worldwide attention to hemicellulose fermentation. Xylan is a major constituent of hemicelluloses. D-Xylose is easily produced by acid or enzymatic hydrolysis of xylan **(Li et al., 2016).**

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