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Pharmacology of Glucose Oxidase Produced by Fungi- Comprehensive Review

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ABSTRACT

Filamentous fungi are known to mainly grow in different environments like organic waste, soil, plant cell material etc. To obtain the nutrients, fungi avails many organic compounds with the aid of various bioenzymes it produce. Glucose-1-oxidase (GOX) (β-D-glucose: oxygen-1- oxidoreductase, EC 1.1.3.4) is an all-around portrayed enzyme, organisms like Penicillium sp, Aspergillus sp has the ability to synthesize the glucose oxidase (GOD).

KEYWORDS: *β-D-glucose, oxygen-1- oxidoreductase, glucose oxidase (GOD), Penicillium sp, Aspergillus sp*

INTRODUCTION

Since 1950's there are a lot of advances in the applications of hydrolytic enzyme, the glucose oxidase (Sorigué et al., 2021). This particular enzyme GOD (EC 1.1.3.4), is purified and developed from a number of sources of fungus like the genus Aspergillus and the genus penicillium. Among them is the mostly used source Aspergillus niger. It avails oxygen which accept electrons to produce gluconic acid by carrying out the -D-glucose β oxidation and at the same time it produces hydrogen peroxide as well. To eliminate glucose from varying sources like the dried form of eggs, mayonnaise to prevent rancidity, fruit juices or beverages and to copious the aroma, expiry, and color of food items, GOD is commercially used. Its application are vast as it is also availed in glucose assay kits and as a biosensor for the assessment and the detection of the glucose in the fluids of the body like urine, the blood etc., and in industrial solutions. Glucose oxidase is effective against various pathogens especially food-borne ones like the Bacillus cereus, Salmonella infantis, Clostridium perfringens, Taphylococcus aureus, the Listeria monocytogens, Campylobacter jejuni etc., . It is also used as a food preservative, to generate gluconic acid on commercial level and in toothpaste, as an important ingredient. The Glucose, oxidase in new preparation with the important

biotechnological application is of a lot of interest to the commercial world (Yoshida et al., 2015).

REACTION MECHANISM OF THE BIOCATALYST GOD

GOD being a flavo-protein and as mentioned earlier it avails its oxygen molecules to accept electrons to produce gluconic acid which, in fact, is the D-glucono-δ-lactone along with the production of hydrogen peroxide. This occurs by the oxidation of the -D-glucose. There are two steps to this reaction: reduction and the oxidation or redox in other words.

The god mechanism especially Glucose-1-oxidase (GOX) (β-D-glucose: oxygen-1-oxidoreductase, EC 1.1.3.4) is an all-around portrayed enzyme, which biocatalyzes the oxidation of β-D-glucose to Dgluconolactone and the hydrogen peroxide. Both hydrogen peroxide and the D-gluconolactone separates suddenly and synergistically. Notwithstanding this current, GOX's bioenzymatic action is lessened when hydrogen peroxide aggregates and inactivates the enzyme; the breakdown result of the Dgluconolactone, gluconic acid ($C_6H_{12}O_7$) gathers, lessening pH of the arrangement. As anyone might expect, both the gluconic acid and the hydrogen peroxide can bring about item hindrance of GOX(Cerutti et al.,2021).

THE STABILITY

The Lyophilized GOD is amazingly steady. At 0°C it is stable for a long time such as 2 years and at -15°C for 8 years duration. In solution the stability is dependent on the pH. It is most steady at around pH 5. Beneath the pH 2 or more pH 8 catalytic movement is quickly lost. At pH 8.1, for instance, just around appox 10% of the action stays after 10 min; at pH 9.1 inactivation is considerably faster. The rate of the inactivation at high pH is decreased in the vicinity of glucose. It is extremely impervious to the proteolysis and is unaffected by delayed exposure to trypsin, the pepsin and papain .Non-ionic detergents have little impact on it, however movement is lost in the vicinity of the ionic detergents like SDS and hexadecyltrimethylammonium bromide. The Anionic detergents like SDS inactivate GOD at low pH and cationic detergents like the hexadecyltrimethylammonium bromide inactivate it at high pH. GOD has a generally low enthalpy of denaturation (precisely 450 kcal mol- ') and is temperamental at temperatures in abundance of 40°C. To some degree it can be mainly ensured against the thermal denaturation by polyhydric alcohols like glycerol. Inhibitors

GOD is restrained by the micro-molar measures of substantial metals, for example, mercury, lead and silver. The enzyme's defenselessness to these metals has been mainly utilized to develop a biosensor intended to distinguish them (Petrović et al., 2017). The Millimolar measures of hydrazine, hydroxylamine and phenylhydrazine somewhat hinder the bioenzyme. At a convergence of 10 mu the accompanying level of the restraint is watched: 8-hydroxyquinoline (11%) sodium nitrate (13%) and the semicarbazide (20%). The movement of the enzyme additionally decreases in the vicinity of the aldohexoses like D-arabinose and 2-deoxy-D-glucose which go about as focused inhibitors. The Halide particles repress GOD at low pH. At pH 3, for instance, it is totally restrained by the 0.1 M potassium chloride. The capacity of polyamines to repress the GOD has as of now been specified (Kommoju et al., 2011).

MICROBIAL PRODUCTION OF GLUCOSE OXIDASE

Commercial sources of the bioenzymes are obtained from three primary sources, i.e., animal tissue, plants and microbies. These naturally occurring bioenzymes are quite often not readily available in sufficient quantities for food applications or the industrial use. However, by isolating microbial strains that produce the desired and precise enzyme and optimizing the conditions for growth, the commercial quantities can be obtained. This technique, well known for more than 3,000 years, is called the fermentation. Today, this fermentation process is carried out in a container vessel. Once the process of fermentation is completed, the microorganisms are mainly destroyed, the enzymes are isolated, and further processed for commercial use. There is a large number of the microorganisms which produce a variety of enzymes. Most of the industrial enzymes are synthesized by a relatively few microbial hosts like Aspergillus and Trichoderma fungi, the Streptomyces fungi imperfecti and Bacillus bacteria. Yeasts are not good produces of the extracellular enzymes and are rarely used for this purpose (Kelley et al., 1988).

After the fermentation process is completed, the bioreactor still contains large amounts of useless nutritive sources, water, microorganisms and the useable enzymes. After the completion of the fermentation process is recovery of the glucose oxidase from the reactor. Glucose oxidase can be synthesized intracellularly, extracellularly, or in the form of mycelia. The separation of the glucose oxidase from cells or mycelia can be facilitated by using mechanical forces, centrifugation and the filtration. Productivity of extracellular glucose

oxidase was examined for various microorganisms and it was found in the strains belonging to genus Penicillium. As the best glucose oxidase producer, the Penicillium purpurogenum No.778 was isolated from natural source. This microorganism produced the glucose oxidase in a simple medium containing beet molasses, $NaNO_3$ and the KH_2PO_4 by submerged culture for 3 days. That value was about 10-times of that of the Penicillium amagasakiense which has been known as an excellent glucose oxidase producer (Patel et al., 2014). The Culture conditions for glucose oxidase production were examined precisely, which were extremely diff erent among microbial species. In the case of the Penicillium chrysogenum AJ7007 and Penicillium purpurogenum No.778, the effects of the aeration and carbon sources were remarkably diff erent from each other. It has also been reported that Penicillium notatum and the Penicillium chrysogenum produce glucose oxidase in the surface culture but not in submerged culture. Glucose oxidase was purified about the 25-fold from culture supernatants of Penicillium purpurogenum No.778, and some properties of the bio-enzyme were examined.

The optimum temperature and pH for the activity were 35°C and 5.0, respectively. The bio-enzyme was stable at pH 5.0 to 7.0 when it was incubated at specifically 40°C for 2 hr, while it was stable at temperature lower than 50°C when incubated at pH 5.6 for 15 min. The Production of GOD by fungi is commonly performed by solid-state fermentation (SSF) and the submerged fermentation (SmF). SmF has been found to be more effective at producing the GOD because it is easier to control the environmental factors involved in this bio-technique when compared to SSF. The studies also explain an SSF process for the low-cost production of the important enzymes with attractive properties for commercial applications.

These research findings confirmed that the A. tubingensis was able to exhibit remarkable GOD activity when culture conditions were precisely optimum as compared to other producer strains. However, these traditional methods for the production of the GOD have reached their limit. Accordingly, different strategies to overcome these limitations and copious their production have been explored, including recombination, the immobilization, mutagenesis and screening (Shin et al., 1993). For the practical applications, immobilization of microorganisms on the solid materials offers several advantages, including repeated usage of enzyme, ease of the product separation and improvement of enzyme stability. Cellulose is a polysaccharide (Sreeremya. S et al., 2014). The polysaccharide has a biopolymer property too

(Sreeremya. S et al., 2017). Many fungi have the ability for cellulose degradation (S. Sreeremya, 2018). Cellulase is the enzyme involved in degradation of cellulose (S. Sreeremya et al., 2018). The cellulase helps in fastening the cellulose degradation (A. K. Midhul et al., 2025).Enzyme like cellulase have biocatalytic property (Dr. S. Sreeremya et al., 2025).

A number of the nutritional factors influencing glucose oxidase (EC 1.1.3.4) production by Aspergillus niger NCIM 545 were refinely studied. Considerable amount of glucose oxidase was produced from the A. niger species with sucrose as the carbon source, sodium nitrate as the inorganic nitrogen biosource, and peptone as the organic nitrogen source. Glucose oxidase activity aggrandized remarkably by 28.93 fold (from 0.00993 to 0.29 U ml−1) with CaCO3 -supplemented media. The outcome of the Plackett–Burman design showed $CaCO_3$, peptone, and MgSO4 as significant parameters. Further optimization availing a three factor central composite design with 20 experiments increased yield of the glucose oxidase from 0.29 to 2.05 U ml−1 (sevenfold) with a decrease in the cultivation time from 96 to 72 h.

Finally, the GOD production was investigated in a semi-continuous system for 7 days. The most frequent isolates, precisely isolated from soil samples belonged to the genera Aspergillus, Penicillium and the Trichoderma. Aspergillus niger LMM01 was the best GOD producer. Glucose, the peptone and KH2 PO4 were demonstrated to be the optimal carbon, nitrogen and phosphorus biosources, respectively. Multivariate experiments demonstrated that the parameters with the greatest effect on the GOD production were pH and agitation (Sing et al., 2008). The Stable expression results for GOD (7.74 U/ml) were obtained over 7 days in a semi-continuous process.

THE APPLICATIONS OF GLUCOSE OXIDASES

Due to constantly aggrandizing level of pollutants governments of many countries imposing stricter limitations on release of the pollutants. Therefore, there is ever increasing demand for clean processes i.e., the processes which either cause no pollution or less pollution (Eryomim et al., 2006). Textile industry particularly the chemical processing sectors always has a major share in the global pollution. BioEnzymes can often replace chemicals or processes that present safety on environmental issues. Enzymes play a pivotal role in such alternative processes (Fig-1,2,&3) .

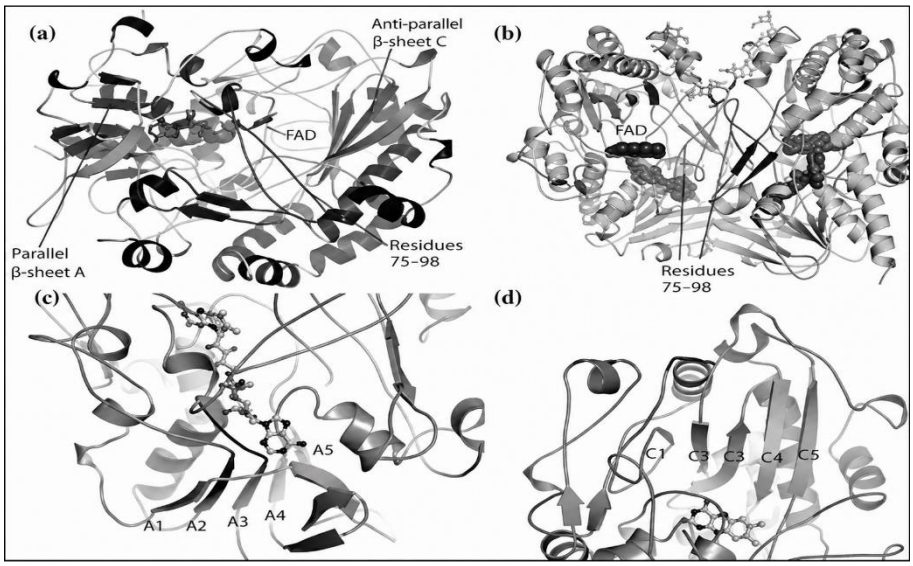

Figure: 1

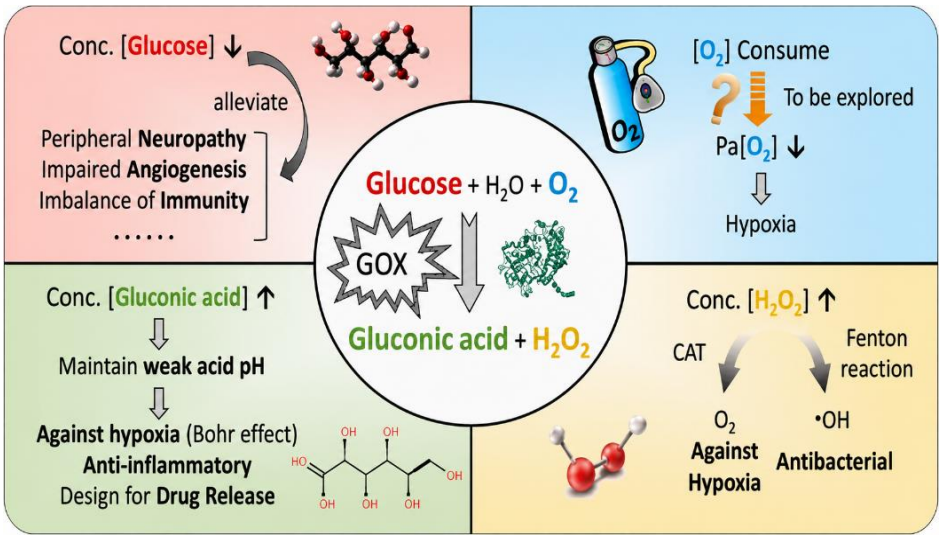

Figure: 2

B. licheniformis	α-amylase, protease
Fungi	
A. niger	Amylases, protease, pectinase, glucose oxidase
A. oryzae	Amylases, lipase, protease
Candela lipolytica	Lipase
Penicillium purpurogenum	Glucose oxidase
Penicillium amagasakiense	Glucose oxidase
Penicillium chrysogenum	Glucose oxidase
Penicillium notatum	Glucose oxidase
Penicillium pinophilum	Glucose oxidase
Penicillium variabile	Glucose oxidase

Figure: 3

MATERIALS AND METHODS –RESEARCH STUDIES

Sample Collection

The Soil samples were collected randomly from a sugar cane market, at the bagasse dumping site, the Donga Local Government, Taraba State Nigeria, during the month of October, 2023.Yam peels were also collected randomly from Block Wukari, Taraba State Nigeria, in the month of October, 2023. The Isolation of fungi Agar (PDA agar) was prepared by weighing 3.9 g of powdered Agar. This was dissolved precisely in 100 ml of distilled water under flame and the autoclaved at 121^{o} C for 5 minutes after which it was kept in the dark and allowed to cool. It was then sealed with the foil paper. 7 test tubes were placed on a test tube rack filled with the 9 ml of distilled water (Kim et al., 1991). 1g of the soil sample was weighed and transferred to the first tube and saturated from the first tube. 1ml of the precise solution was transferred to the test tubes. This process was repeated until the last tube was precisley 10ml.4 plates were selected and 0.1ml of the diluted sample was specifically placed in the plate and covered care was taken in the stage as the plates were specifically labeled with the name of the dilutions (plate 1; 10^{-1}, plate 2; 10^{-2}, plate 3; 10^{-3} and so on). 0.1ml of the soil dilution of 10-1 was poured into the plate labeled 10-1 and 10ml of the soil dilution of 10-2(Kim et al., 1990). This was due until the 7th plate containing the 10-6 dilution solution was covered immediately. About 15-20 ml of the Agar was poured into the plates labeled 10-1 to 10-6 and swirled gently to avoid splash at the surface of the plates (Stosz et al., 1998). The plate was mainly supplemented with Streptomycin to avert bacterial contaminations.

Finally, the plate was specifically allowed to solidify then inverted and incubated for 24 hours at 39 ^{o} C. The Subculture of enzyme-producing microorganism the Agar solution was prepared by weighing 3.9 g of the Agar pounder and dissolved in 100 ml of the distilled water under flame, autoclaved at 121°C for 15 minutes after which it was kept on the desk and slowly allowed to cool. It was sealed with a foil paper (autoclaving was done availing conical flask). The Agar solution was contained in a conical flask before introducing to the autoclaving chamber). After the Agar has cooled, it was poured into the 6 plates still labelled with (10-1 to 10- 6) and allowed to solidity. Following the observation from the phase experiment, it was discovered that the plate 2 (10-1), 4 (10-3) and 6 (10-5) had a good colony condition among the others. After the subsequent process (Gould, 1975), A brown coloured zone around the medium was observed while the colony grew only on the middle of the plate.

Fermentation for the glucose oxidase production the fermentation medium for the production of the glucose oxidase was containing the following chemicals. The Glucose, peptone, $(NH_4)_2HPO_4$, KH_2PO_4, $MgSO_4$.$7H_2O$ and $CaCO_3$. 14 The preparation of the medium was done in percentages for the second batch. The 3 conical flasks containing the basal medium were prepared. The 8% Glucose, 0.3% peptone, 0.038% $(NH_4)_2$ HPO_4 , 0.0188% KH_2 PO_4 , the 0.0156% $MgSO_4$.$7H_2O$, 3.5% $CaCO_3$ was required in the 100ml of distilled water since the nutrient source are expressed in percentages and a conical flask was needed for the carbon source. A total of almost 24g of Glucose, 0.9g peptone, 0.1164g of $(NH_4)_2HPO_4$, precisely 0.0564g of KH_2PO_4, 0.0468g of $MgSO_4.7H_2O$ and 10.5g of $CaCO_3$ were weighed into a clean conical flask and a total of 300ml of distilled water was availed to dissolve the chemical nutrients. This produced a milky coloration. 50ml of the basal medium was poured into a clean conical flask and 30g of yam peel was added to the conical flask to make it moist. The conical flask was mainly autoclaved at 121^oC for 15 mins to remove the contaminations after which a loopful of A. niger was inoculated into the asceptically maintained conical flask and tightly sealed and allowed to ferment for 5 days (Karmali et al., 2004).

CONCLUSION

The bioenzymes have many functionalities and it has varying applications. The role of bioenzymes from the microbial source has a plethora of possibilities. There are microorganisms especially fungi like *Aspergillus sp, Penicillium sp* which biocatalyzes the oxidation of β-D-glucose to Dgluconolactone and the hydrogen peroxide .

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