EXTRACTION OF GLUCOSE ISOMERASE FROM *STREPTOMYCES* SP. AND ITS ACTIVITY

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Abstract

The present study is concerned with the screening and isolation of glucose isomerase producing *Streptomyces* species from soil. Eleven soil samples were collected from the University of Yangon Campus (YU) and Pharmaceutical Research Department Campus (PRD), Yangon, Myanmar. A total of 29 strains of microorganisms were isolated and their glucose isomerase activites were screened by two different methods such as plate assay method by using three different media; X^+P^+, X^+P^- and wheat bran, and by fructose estimation method by using Seliwanoff's reagents. Among these 29 strains, only four strains: YU1-MAN3, YU3-PNI42, YU3-PNI44 and YU7-RCC3 showed glucose isomerase activity, and two strains YU3-PNI42and YU3-PNI44 were characterized as *Streptomyces* sp. according to the morphology, Gram's staining, spore staining, catalase test, gelatin test and nitrate test. The activities of glucose isomerase enzymes GI-PNI42 and GI-PNI44, extracted from *Streptomyces* sp. YU3-PNI 42 and YU3-PNI 44, were found to be 0.070 μmol min^{-1} mL^{-1} and 0.052 μmol min^{-1} mL^{-1}, respectively.

Keywords: *Streptomyces* sp., glucose isomerase, plate assay, wheat bran

Glucose isomerase (GI) enzyme is considered as one of the most important industrial enzymes (Sriprapundh *et al.*, 2003; Rao*et al.*, 2008) and it is the third highest value enzymes after amylase and protease (Srivastava *et al.*, 2010). The main practical application of this enzyme for its ability to isomerize D-glucose to D-fructose, and hence, it is widely used in industry for production of high-fructose corn syrup (HFCS) which is used all over the world as an alternative to sucrose or invert sugar in the food and beverage industry (Fenn *et al.*, 2004;Heo *et al.*, 2008; Brat *et al.*, 2009). Glucose isomerase (GI) can be isolated from a wide variety of bacteria such as Osmophillic *Aspergillus* sp. (Sayyed*et al.*, 2010), *Aerobacter aerogenes*, Strain HN-56 (Natake and Yoshimura, 1963), *Enterobacter agglomerans* (Nobel Surya Pandidurai *et al.*, 2011) and *Saccharamyces cerevisiae* (Brat *et al.*, 2009). Among them, *Streptomycetes* is one of the preferred categories of

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organisms for production of this enzyme. *Streptomycetes* are filamentous bacteria which possess extensive genes of primary and secondary metabolism. They are present in soil in abundance and are responsible for the degradation of all kinds of organic matter. This efficiency can be accounted by their capacity to produce different types of enzymes; amylase, protease, lipase, cellulase, glucose isomerase, xylanase, pectinase etc.

Some of the research for producing GI from *Streptomyces* sp. strain C (Bandlish *et al.*, 2002), *Streptomyces murinu* (Al-Tai *et al.*,1987), *Streptomyces phaeochromogenes* (Tsumura *et al.*, 1965), *Streptomyces albus* (Sanchez and Smiley, 1975), *Streptomyces* sp. (Takasaki, 1966), *Streptomyces cinnamonensis* (Joseph *et al.*, 1977), *Streptomyces flavovirens* (Sriprapundh *et al.*, 2003), *S. fradiae* (Mand *et al.*, 1977), *Streptomyces lividans* TK24 (Heo *et al*., 2008) and *Streptomyces* sp. (Chou *et al.*, 1976) had been published. However, the continuous studying of GI production from *Streptomyces* species is still being studied all around the world to achieve better GI producers.The present study is concerned with the screening and isolation of glucose isomerase producing *Streptomyces* species from soil.

Materials and Methods

Chemicals and Materials

All chemicals used in this research were from Merck, Germany. The materials are sterilizer (SM 310 Yomato), hot air sterilizer (GM-10E, DRWG No. YB-81051, Hawirawa Works LTD.), clean bench (CCV-1301EC, DRWG No. 4B-81048, Hitachi LTD.), medical freezer (Sanyo), microscope (Nicon, Japan), analytical balance (ATX224, Shimadzu), touch mixture (MT-11, Yomato), refrigerated centrifuge (5500, KuBoTa), incubator (Sanyo), homogenizer (HG-150, Wisetis), UV-Vis spectrophotometer (cenesys10s uv-vis, Thermo scientific), Shaking incubator (Jisico), digital water bath (ΔLab Tech, Dai Han lab tech Co. LTD.), loop, needle and, glasswares.

Soil Sample Collection and Isolation of Microorganisms

A total of 11soil samples were collected; 9 from the University of Yangon Campus (YU) and 2 from Pharmaceutical Research Department Campus (PRD), Yangon, Myanmar. One gram of each soil sample was 10 fold serially diluted in sterile water and 0.1 mL of each solution from dilutions

of 10^{-4} –$10^{-6}$was then spread on Peptone Yeast Agar medium plate (1 % peptone, 0.5 % yeast extract, 1 % xylose, 0.3 % K_2HPO_4, 0.1 % $MgSO_4$. $7H_2O$ and 2 % agar at pH 6.8–7 by the techniques of Dubey and Maheshwari (2009). Well grown colonies were picked and further purified by streaking onto the same agar medium plate. The pure strains were stored on Peptone Yeast Agar medium slant.

Preliminary Screening of Glucose Isomerase Producers

The isolates were screened qualitatively on xylose and wheat bran media. The organisms producing glucose isomerase can isomerise xylose to xylulose besides glucose to fructose. The organisms possessing very low or negligible GI activity might not grow on such a media. The screening strategy was designed according to the method described by Manhas and Bala(2004) and the modified method by Sheetal *et al.*(2013).Three different media, X^+P^+agar medium (1 % xylose 0.03 % peptone, 0.2 % KNO_3, 0.2 % K_2HPO_4,0.2 % NaCl, 0.05 % $MgSO_4.7H_2O$, 0.01 % $FeSO_4$, 0.02 % $CaCO_3$, 2 % agarat pH 7), X^+P^-agar medium (without peptone) and wheat bran agar medium (wheat bran was substituted for xylose as a carbon source) were used for preliminary screening. The cultures were spot inoculated on all the media and incubated at 30°C. The early developing isolates within 24 hron the plates were considered as higher GI producers.

Preparation of Enzyme Extracts

Peptone Yeast broth medium was used as a culture developing medium. The organism was grown in the medium at 30 °C for 4 days. The culture was harvested and centrifuged at 2000 rpm for 20 min at 4 °C. The cell was then collected and washed with potassium phosphate buffer (pH 7). After that, the cell was suspended with potassium phosphate buffer (pH 7). The cells were disrupted with the speed of 400 rpm for 5 minby Homogenizer. The cell free supernatant as crude intracellular enzyme extract was collected by centrifuging at 2000 rpm for 20 min at 4 °C.

Confirmation of Glucose Isomerase Extracted enzymes

The glucose isomerase activity was confirmed by Seliwanoff's reagent. The reaction mixture, according to Sathya and Ushadevi (2014)**,**

containing 0.4mL of crude enzyme and 0.6 mL glucose solution (0.1 M) was made up 2 mL with distilled water and incubated at 75 °C for 30min. Then, 2 mL of Seliwanoff's reagent was added into the reaction mixture and heated for 5min. The formation of cherry red colourindicated the occurrence of fructose due to the glucose isomerase activity of the test culture.

Identification of *Streptomyces* Sp.

The microorganism producing glucose isomerase enzyme was selected and further identified as a *Streptomyces* sp. The physiological and biochemical characteristics were determined. The colour and mycelium producing were detected on the growth of culture. Gram's staining, spore staining, citrate utilization, gelatin liquefaction, catalase and nitrate reduction were tested(Buchannan and Gibbons,1974).

Characterization of Glucose Isomerase Extracted from *Streptomyces* Sp.

The reaction mixture contained 0.2mL of 1 M glucose, 0.5 mL of potassium phosphate buffer, 0.1mL of 0.1M $MgSO_4.7H_2O$, 0.1mL of 0.01 M $CoCl_2$ and 0.2mL of enzyme extract. The final volume of assay mixture was made up to 2 mL with distilled water Isomerization was carried out at 70 °C for 30 min in water-bath. The reaction was stopped by adding 2 mL of 0.5 M perchloric acid. The fructose produced by isomerization was determined by the method described by Dische and Barnfoed (1951).To an aliquot of 0.1 mL of the above mixture, 0.9 mL of distilled water was added. 0.2 mL of 1.5 % cysteine hydrochloride, 6 mL of 70 % sulphuric acid and 0.2 mL of 0.12 % alcoholic carbazole were then added. The intensity of purple colour developed was determined at 560 nm using standard curve. One unit of glucose isomerase activity was defined as the amount of the enzyme that produced 1 μmol of D-fructose per minuteunder the assay conditions described.

Results and Discussion

Screening of Glucose Isomerase Activity of Isolated Microorganisms

A total of 29 culture strains were isolated from the soil. The glucose isomerase activity of isolated strains were primarily screening on three different media(X^+P^+ medium, X^+P^- medium and wheat bran medium). The

GI producers were characterized by two factors. One is the growth rate on the media; the higher growth of strains produced higher GI activity and the lower growth rate showed lower GI activity. And the other is the growth in X^+P^+ and X^+P^- media; the strain grown inonly $X^+P^+$was assumed as moderate GI producer and one grown inboth X^+P^+ and $X^+P^-$is good GI procedure. All strains grew in wheat bran medium but only 8 strains showed growth rate activity on X^+P^+ and X^+P^- media. The isolates giving early appearance on wheat bran medium also grew well on other two media. X^+P^+ and X^+P^- media gave a clear picture of isolates as GI producers (Sheetal *et al.*, 2013).The number of (+) signs is directly proportional to the growing capacity of the isolate on media. A comparison of growth on all the above media is shown in Table 1.This process indicated that 8 isolated strains were GI producers. The cultures selected by this method were further screened by Seliwinoff's reagent. The glucose isomerase enzymes were extracted from the selected culture.

Table 1: Growth Rate of Various Isolated Microorganisms on different Media

No.	Strains	Growth rate of microorganisms		
		X^+P^+	X^+P^-	Wheat Bran
1	YU1-MAN3	++	+++	+++
2	YU1-MAN21	+	–	+++
3	YU2-PNN3	+	+	+++
4	YU3-PNI42	+++	+++	+++
5	YU3-PNI44	+++	+++	+++
6	YU6-RCB5	+	–	+++
7	YU7-RCC3	++	+	+++
8	YU8-O7	+	–	+++

(+++) High growth rate, (++) Moderate growth rate, (+) Low growth rate and (–) No growth rate

The enzymatic reaction (isomerization of glucose to fructose) was performed using glucose as a substrate and fructose was identified by Seliwanoff's reagent. The cherry red colour development was identified against standard and blank test sample. In this process, among selected 8

strains, only 4 strains; YU1-MAN3, YU3-PNI42, YU3-PNI44 and YU7-RCC3showed higher growth rate inX^+P^+ and X^+P^- were found to be the similar colour forming as standard. The strains with very lower rate in screening plate media demonstrated negative glucose isomerase activity and the results are illustrated in Table 2. These methods are highly suitable for screening of glucose isomerase activity of microorganisms.

Table 2: Screening of Glucose Isomerase Activities of Enzymes Extracted from Various Isolated Microorganisms

No.	Strains	Extracted Enzymes	Glucose Isomerase Activities
1	YU1-MAN 21	GI-MAN 21	–
2	YU1-MAN3	GI-MAN3	+
3	YU8-O7	GI-O7	–
4	YU6-RCB5	GI-RCB5	–
5	YU7-RCC3	GI-RCC3	+
6	YU3-PNI42	GI-PNI42	+
7	YU3-PNI44	GI-PNI44	+
8	YU2-PNN3	GI-PNN3	–

(+) Glucose isomerase activity observed; (–) No glucose isomerase activity observed

Identification of *Streptomyces* Sp.

The four strains produced GI activity were selected and characterized based on their morphological, physical, cultural and biochemical properties with the help of Bergey's Manual of Determinative Bacteriology (Buchannan and Gibbons, 1974) and the Actinomycetes Vol. II (Selman and Waksman, 1961).Two of these species, YU1-PNI42 and YU1-PNI44 (Figure 1) were found to be Gram positive and spore positive, which is one of the important criteria of the *Streptomyces* sp.

(a) YU3-PNI42

(b) YU3-PNI44

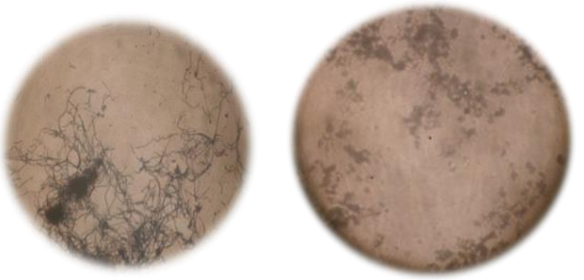
Figure 1: Pure culture *Streptomyces* sp.

These strains were studied morphologically and microscopically. Microscopic observation revealed that only YU1-PNI42 and YU1-PNI44 showed better performance in the production of mycelia as well as sporulation (Figure 2). Other two strains, YU1-MAN3 and YU7-RCC3 could not produce mycelium but both were rod-shape. Therefore, according to the results and theoretical approach, it could say that YU1-MAN3 and YU7-RCC3 were not *Streptomyces* sp. and further consideration of the results of these strains could be neglected in the research. A biochemical characteristics of the selected isolates; such as catalase, gelatin, and nitrate had been considered for the identification of *Streptomyces* sp. analyzed (Table 3).

Table 3: Results of Characterization of *Streptomyces* Sp.

No.	Tests performed	Observation			
		YU3-PNI44	**YU3-PNI42**	**YU1-MAN3**	**YU7-RCC3**
1	Surface colour	watery	brown	Creamy	Creamy
3	Shape	mycelium	mycelium	Rod	Rod
5	Shape in liquid medium	pearl shaped	pearl shaped	–	–
6	Gram's stain	+	+	+	+
7	Spore stain	+	+	–	–
8	Gelatin	+	+	+	+
9	Catalase	+	+	+	+
10	Nitrate	+	+	+	+

+ = positive and– = negative



(a) (b)

Figure 2: Microscopic observation of *Sterptomyces* sp. (a) Mycelium (b) spore forming

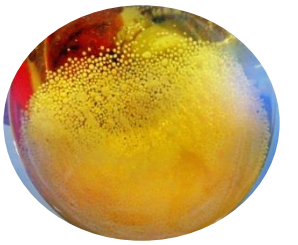


Figure 3: 4 day ages-*Sterptomyces* sp. in broth medium

Nitrate reduction, gelatin liquefaction and catalase tests were found to be positive in case of all strains. Other theoretical information was considered to this research to identify *Streptomyces* sp. In this research, the strain YU3-PNI42 and YU3-PNI44 was found that the growth rate was slow when they were incubated in at 37 °C butgrow well at 30 °C. Theoretical growth temperature range of *Streptomyces* sp. was 28 °C–30 °C. Streptomyces being a filamentous bacterium grew as pearl-shaped in the liquid medium.YU3-PNI42and YU3-PNI44 illustrated the pearl-shaped (Figure 3) in liquid medium and the beads grew in size and number with increase in incubation time.

Enzyme activity of Extracted GI from Isolated *Streptomyces* sp.

The activity of the glucose isomerase enzyme extracted from the strains of YU3-PNI42 and YU3-PNI44 was determined by isomerization of glucose substrate to fructose. The absorbance fructose formed was measured at 650 nm. The activities of glucose isomerase enzymes GI-PNI42 and GI-PNI44, extracted from respective strains were found to be 0.070 µmol $min^{-1}mL^{-1}$ and 0.052 µmol $min^{-1}mL^{-1}$, respectively. *Streptomyces* sp., YU3-PNI42 was found to be high glucose isomerase producer and the activity of enzyme of GI-PNI42 from YU3-PNI42 was higher than GI-PNI42 from YU3-PNI44 (Table 4).

Table 4: Activities of Glucose Isomerase Enzyme Extracted from the Isolated *Streptomyces* sp.

Enzyme Solutions	Enzyme Activity ($\mu mol\ min^{-1}\ mL^{-1}$)
GI-PNI42	0.070
GI-PNI44	0.052

Conclusion

The 11 soil samples were collected and cultured on PYA medium by serial dilution method. The pure colonies were isolated. Then the 29 pure cultures were screened on three different media plate such as X^+P^+, X^+P^- and wheat bran media. The 8 culture growth on these media was primarily selected and their GI activities were further confirmed by Seliwanoff's reagent. The strains grew with the very low growth rates in plate media did not show its GI activity when confirmed by Swellinoff's reagent. The four strains: YU1-MAN3, YU3-PNI42, YU3-PNI44 and YU7-RCC3 showed Glucose Isomerase activity. Only two strains: YU3-PNI42 and YU3-PNI44 could be characterized as *Streptomyces* sp. by assay methods. The activities of glucose isomerase enzymes GI-PNI42 and GI-PNI44, extracted from *Streptomyces* sp. YU3-PNI42 and YU3-PNI44, were found to be 0.070µmol $min^{-1}mL^{-1}$ and 0.052µmol $min^{-1}mL^{-1}$, respectively.

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