



Production of Glucose Isomerase from *Streptomyces roseiscleroticus*

**Okwuenu Prosper Chinyelum^{1*}, Onosakponome Iruogene¹
and Oparaji Emeka Henry¹**

¹Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka,
Enugu State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2021/v21i1030396

Editor(s):

(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.

Reviewers:

(1) Uraiwan Intamaso, Burapha University, Thailand.

(2) Amr El-Waseif, Al-Azhar University, Egypt.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/72684>

Original Research Article

**Received 08 June 2021
Accepted 17 August 2021
Published 22 October 2021**

ABSTRACT

This study was aimed at the isolation and characterization of a microbial strain capable of producing glucose isomerase. Microbial strain was isolated from soil using starch casein agar as a differential media. The isolated microbial strain was capable of producing glucose isomerase which was tested using 2, 3, 5 - triphenyltetrazolium solution as a chromogenic substrate. The microbial strain was identified as *Streptomyces* species based on its morphological and microscopic characteristics. It was further subjected to molecular characterization using 16S rRNA sequencing and was subsequently confirmed as *Streptomyces roseiscleroticus*. Glucose isomerase was produced from *Streptomyces roseiscleroticus* after 120 hr of submerged fermentation at pH 6.8 and at 37°C utilizing xylose as the sole carbon source and a compendium of peptone, beef and yeast extracts as nitrogen sources. These findings suggest that the microbial strain, *Streptomyces roseiscleroticus* can be a useful bacterial source for the production of glucose isomerase needed for commercial and industrial utilization.

Keywords: *Streptomyces roseiscleroticus*; glucose isomerase; 16S rRNA; sequencing; submerged fermentation.

1. INTRODUCTION

Glucose isomerase (EC.5.3.1.5) is predominantly an intracellular enzyme [1] that catalyzes the reversible isomerization of D-glucose to D-fructose [2]. This enzyme is an oxidoreductase that interconverts an aldose and a ketose [3]. Glucose isomerase belongs to the enzyme classification of the Isomerases, to the group of the Intramolecular oxidoreductases, and to the sub group of enzymes, interconverting in the sugar series, aldoses to ketoses, ketoses to aldoses and other related compounds [4].

The isomerization ability of glucose isomerase is characterized by a hydride shift mechanism which involves ring opening of the substrate, isomerization via a metal ion induced hydride shift from carbon-2 to carbon-1 of the substrate, followed by the ring closure of the product formed [4 and 5].

The isomerization ability of glucose isomerase has attracted great attention because of its usefulness in the production of sugar syrups such as fructose syrup and sugar substitutes which is used world over as an alternative to sucrose or invert sugar in the food and beverage industries [6]. This has made glucose isomerase applicable in a number of food processing industries and biotechnological processes [7]. Apart from the food industry, this enzyme has recently gained more interest due to its potential applications in the biofuel industry majorly in ethanol production [8]. Glucose isomerase is a tetramer composed of four identical polypeptide chains or a dimer of related subunits. Each monomer consists of two domains: a large N-terminal domain and a small C-terminal loop [9].

Many microorganisms have been reported to produce glucose isomerase, microorganisms especially the anaerobic *clostridium*, *thermosulfurogenes*, *thermoanaerobacter*, *thermoanaerobacterium*, aerobic *pseudomonas*, *bacillus*, *streptomyces*, alkalophilic and acidophilic *actinomycetes* [10]. However, glucose isomerase from the *bacillus* species, *streptomyces* species, *actinoplanes* and *arthrobacter* species have been reported to be most suitable for commercial, industrial and economic utilization [11,6] because of their ease of isolation and characteristics of being manipulated by genetic engineering and biotechnological techniques [12, 1].

Streptomyces species have been reported to be the microorganism of choice for glucose isomerase production, this is because, they have very high potential to produce secondary metabolites [13], such as antibiotics, enzymes [14], anti- cancer drugs [15], growth factors like vitamin B12 [16] and immune- modulators [17]. Some members of *Actinomycetes* group like *Actinomyces olivocinereus*, *Actinomyces phaeochromogenes*, *Actinoplanes missouriensis*, have been reported to produce good amounts of glucose isomerase. Other bacteria, such as *Bacillus stearothermophilus*, *Bacillus megabacterium*, *Bacillus coagulans*, *Bifidobacterium* species, *Brevibacterium incertum*, *Bacillus pentosoaminoacidicum*, *Lactobacillus brevis*, *Lactobacillus buchneri*, *Lactobacillus fermenti*, and *Lactobacillus bifermantans* are also reported to produce glucose isomerase [6]. There are reports of glucose isomerase being extracellular as well as intracellular [18], however, reports on extracellular secretion of glucose isomerase are not common. Extracellular glucose isomerase has been reported to be produced by *Streptomyces glaucescens* and *Streptomyces flavogriseus*, for which the enzyme secretion from the cells was attributed to a change in the cell wall permeability and partial lysis of the cells [18]. Also, the occurrence of glucose isomerase in a few yeasts such as *Candida utilis* and *Candida boidinii* has been reported [2]. *Aspergillus oryzae* is the only known glucose isomerase producing fungus reported [4 and 19]. Glucose isomerase is reported to be abundantly produced by a fermentation process, particularly, submerged aerated fermentation [1].

This research work was therefore aimed at the isolation and characterization of a microbial strain capable of glucose isomerase production.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents Used

All chemicals used in this research were of analytical grade and were obtained directly from the manufacturers. Major chemicals such as 2, 3, 5 - triphenyltetrazolium solution, carbazole, cysteine hydrochloric acid, Bovin serum albumin (BSA), dodecyltrimethylammonium bromide (DTAB), Folin- ciocalteau reagent and starch casein agar were obtained from Sigma Aldrich, Germany.

2.1.1 Collection of soil sample and isolation of microorganism

Soil sample was obtained from the Department of Soil Science garden, University of Nigeria, Nsukka, using a sterile container. Isolation of the microorganism was done using the method explained by Silva *et al.*, [20]. A known weight of the soil sample (1 g) was mixed with distilled water (10 ml.). A volume (100 µl) of the solution was poured on Petri dishes containing nutrient medium (KH₂PO₄ 0.02%, starch 0.1%, casein 0.04%, MgSO₄.7H₂O 0.01%, KNO₃ 0.05%, CaCO₃ 0.01% and agar 1.5%) mixed with 500 mgL⁻¹ of penicillin. The collected sample was serially diluted up to 10⁻⁵ fold using the spread plate method. The plates were incubated for seven days at 30 °C. The pure isolate was identified using the method of Robert *et al.* [21]. Finally, the bacterial strain was maintained on sterile nutrient agar.

2.1.2 Screening for glucose isomerase producing organism

Glucose isomerase producing bacteria were screened using the method described by Sapunova *et al.*, [22], in which 2, 3, 5 - triphenyltetrazolium solution was used as the chromogenic substrate.

2.1.3 Molecular characterization of the microbial isolate

Extraction and Amplification of Microbial DNA using PCR Genomic DNA (gDNA) was extracted using *AccuPrep* DNA extraction Kits (Bioneer, USA) by following the manufacturer's guidelines. The 16S rRNA of the DNA extract from the bacterial isolate was amplified using the primer pairs, forward primer (5'-AGAGTT TGATCC TGGCTC AG-3') and reverse primer (5'-GCG CTT TTT GAG ATT CGC TC-3'). The primers used in this study was synthesized by Eurofins MWG Operon LLC (Louisville, Kentucky, USA). The PCR mixture contained, 16 µl of nuclease-free water, 2 µl of primer (1 µl of the forward primer and 1 µl of reverse primer), and 2 µl of DNA extract. The reaction mixture was subjected to pre denaturation (5 min, 95°C), denaturation (1 min, 94°C), annealing (1 min, 52 °C), extension (7 min, 72°C) and final extension (7 min, 72°C).

2.1.4 Agarose gel Electrophoresis and DNA sequencing

This was done using 1.5 % agarose gel (1.5 g of agarose in 100 ml of Tris-acetate-EDTA (TAE) buffer. Agarose was dissolved by microwaving in

1 x TAE buffer. The solution was cooled to 55°C and then mixed with 12 µl ethidium bromide. DNA ladder (6 µl) and the amplicon (10 µl) were introduced into the wells of the gel followed by electrophoresis at 100 V for 1 hr. The DNA bands were pictured under a UV light gel imaging system. The amplicon was sequenced using Big dye terminator version 3.1 cycle sequencing kit (Invitrogen, USA)

2.2 Bioinformatics Analysis

The sequenced PCR product was subjected to BLASTn search on the NCBI website to determine its sequence similarity with other bacterial species. Sequences with the highest similarity were extracted and the phylogenetic tree constructed using Molecular Evolutionary Genetics Analysis (MEGA) version 7.

2.3 Glucose Isomerase Production

Submerged fermentation (SmF) technique was used to monitored glucose isomerase production as described by Habeeb *et al* [1]. A volume of 350 ml of the fermentation broth for glucose isomerase production was constituted in an Erlenmeyer flask. The fermentation broth for the enzyme production contained 7 g peptone, 7g D-xylose, 1.75 g yeast extract, 3.5g NaCl, 3.5g beef extract and 0.35g MgSO₄.7H₂O with the pH adjusted to pH 6.8. From the 350 ml of the broth, 50 ml each were poured into seven (7) 250 ml conical flasks, each labeled (one to seven). These flasks were stopped with aluminum foil and autoclaved at 121 °C for 15 min to ensure sterility. The flask were allowed to cool and inoculated with the *Streptomyces* isolate using a 10 mm diameter cork borer. For each day and for each flask, the microbial biomass was separated by centrifugation at 10000 rpm for 15 min and washed severally with distilled water. The cells were further suspended in 0.05 M sodium phosphate buffer pH 7.0 containing 0.1% dodecyltrimethylammonium bromide (DTAB) and then incubated at room temperature for 24 h, after which it was centrifuged and filtered. Protein concentration was determined and glucose isomerase activity was assayed on the filtrate.

2.4 Glucose Isomerase Assay

Glucose isomerase was assayed as described by Habeeb *et al.*, [1]. An aliquot (0.2 ml) of the crude enzyme was added into a mixture of 0.5 ml of 0.1 M potassium phosphate buffer pH 7.5, 0.2 ml of 0.05 M MgSO₄.7H₂O and 0.2 ml of 1 M

glucose solution. After incubation for 30 min at 60 °C in a water bath, 1 ml of 0.5 M of perchloric acid was introduced to stop the reaction. The quantity of fructose produced was determined by the method of Dische and Borenfreund [23] in which 1.5% cysteine hydrochloride (0.2 ml), 70% H₂SO₄ (6 ml) and 0.12% alcoholic carbazole (0.2 ml) were added to the reaction mixture. The purple color formed was measured at 560 nm using a spectrophotometer. The absorbance was converted to glucose isomerase activity using a fructose standard

2.5 Protein Concentration

The protein concentration of the crude enzyme was determined using the method of Lowry *et al.*, [24] and Bovine Serum Albumin (BSA) as standard.

3. RESULTS AND DISCUSSION

Starch casein agar was utilized as a differential medium for the isolation of the microbial strain. The isolated strain was screened for glucose isomerase producing ability using 2, 3, 5 - triphenyltetrazolium solution as the chromogenic substrate (Plate 2). The basis of the plate method screening for glucose isomerase producing ability of microorganism on agarized media lies in the capacity of D-fructose formed by the microbial strain to oxidize the colourless 2, 3, 5 - triphenyltetrazolium chloride in alkaline medium, with the formation of formazan having a cherry red or a rose-red or dark pink coloration of the surface of the microbial colonies or isolates

[22]. The microbial isolate was identified as *Streptomyces* species based on their morphological and microscopic characteristics (Table 1). The *Streptomyces* colonies when plated on nutrient agar were observed to be sporulated, white to milky in colour and grows singly, in clusters and sometimes, linearly (Plate1). The isolated microorganism was a gram positive cocci, and tested positive to catalase, citrate utilization, nitrate reduction and sugar/starch fermentation tests (Table 2).

The isolated *Streptomyces* strain was further subjected to molecular characterization. Agarose gel electrophoresis of the PCR products showed a single amplicon band which corresponds to 780 - 789 bp when compared to the DNA ladder (Fig. 1).

The amplicon band of Lane 11 of the microbial isolate indicates that the isolate very possibly belongs to or is a very close relative to the *Streptomyces* genus. The phylogenetic analysis confirmed that the *Streptomyces* isolate is very closely related to *Streptomyces roseiscleroticus*. (Fig. 2).

Also, from the NCBI blast result of the query sequence (Table 3) and as shown on the phylogenetic tree of evolutionary history (Fig. 2), the *Streptomyces* isolate identified as *Streptomyces roseiscleroticus* had a similarity percentage or bootstrap score of 99.51 % to *Streptomyces roseiscleroticus* strain AS 4.186 with an accession number NR112381 (Table 3).

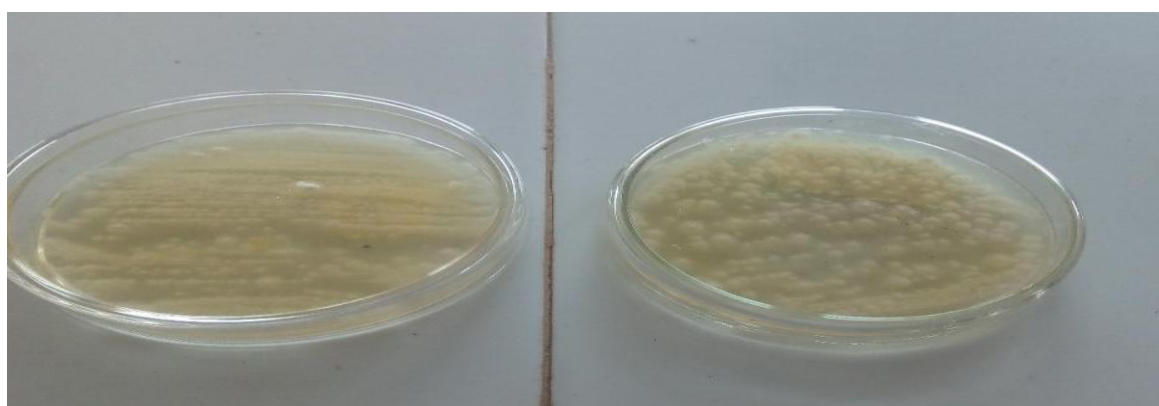


Plate 1. Two day old pure isolates of *Streptomyces* species obtained from soil by serial dilution. The *Streptomyces* colonies were observed on nutrient agar

Table 1. Morphological characteristics of the isolated microorganism on starch casein agar

Shape	Edge	Elevation	Consistency	Smell	Opacity	Colour
Circular or cocci	Round	Raised	Singly	Earthy	Clear	Cream white

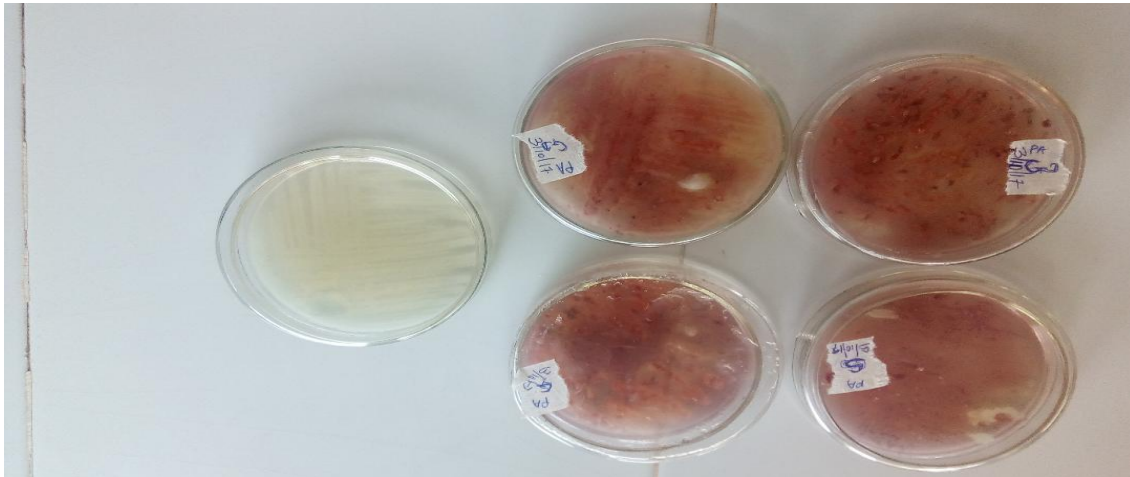


Plate 2. Screening of the microbial strain for glucose isomerase producing ability; Glucose isomerase producing ability was screened using 1, 2, 3-triphenyltetrazolium solution and confirmed by the formation of a cherry red or a rose-red coloration of the surface of the microbial colonies or isolates

Table 2. Biochemical identification of the isolated microorganism

Tests	Results
Gram staining	Gram positive cocci
Citrate utilization	Positive
Nitrate reduction	Positive
Catalase	Positive
Starch hydrolysis	Positive
Sugar fermentation test (glucose, xylose, Galactose, fructose)	Positive

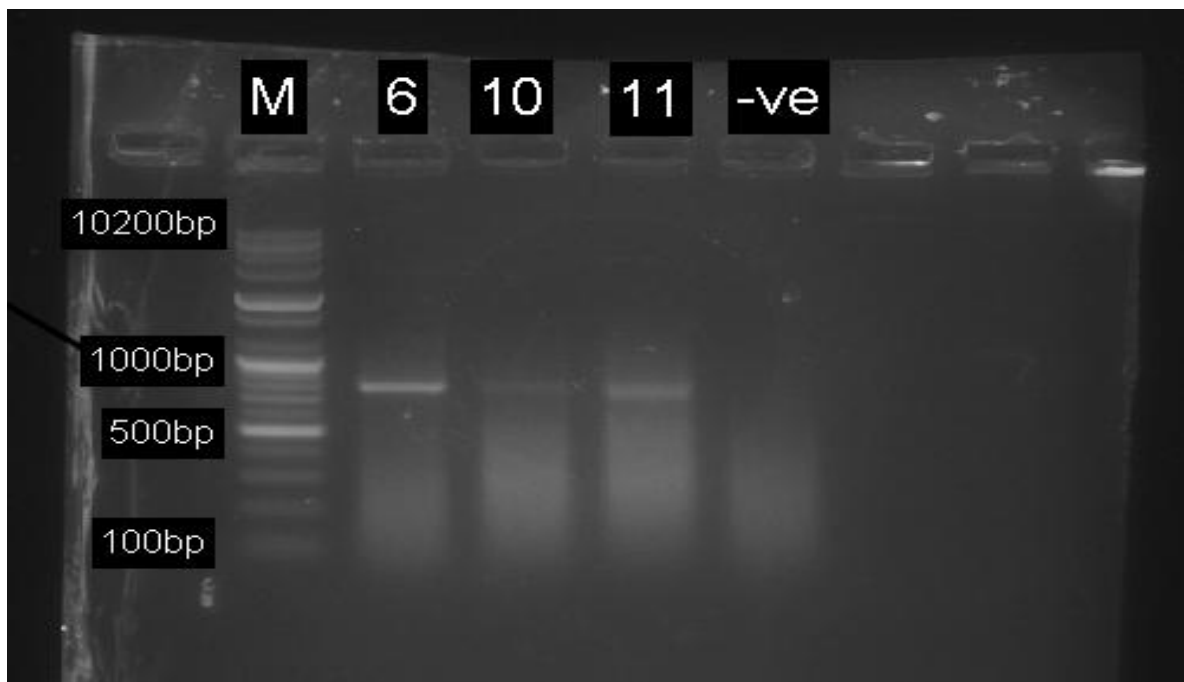


Fig. 1. Agarose gel electrophoresis of PCR products. Lanes M and 11 represents the DNA ladder and *Streptomyces* spp 16S rRNA gene amplicon, respectively

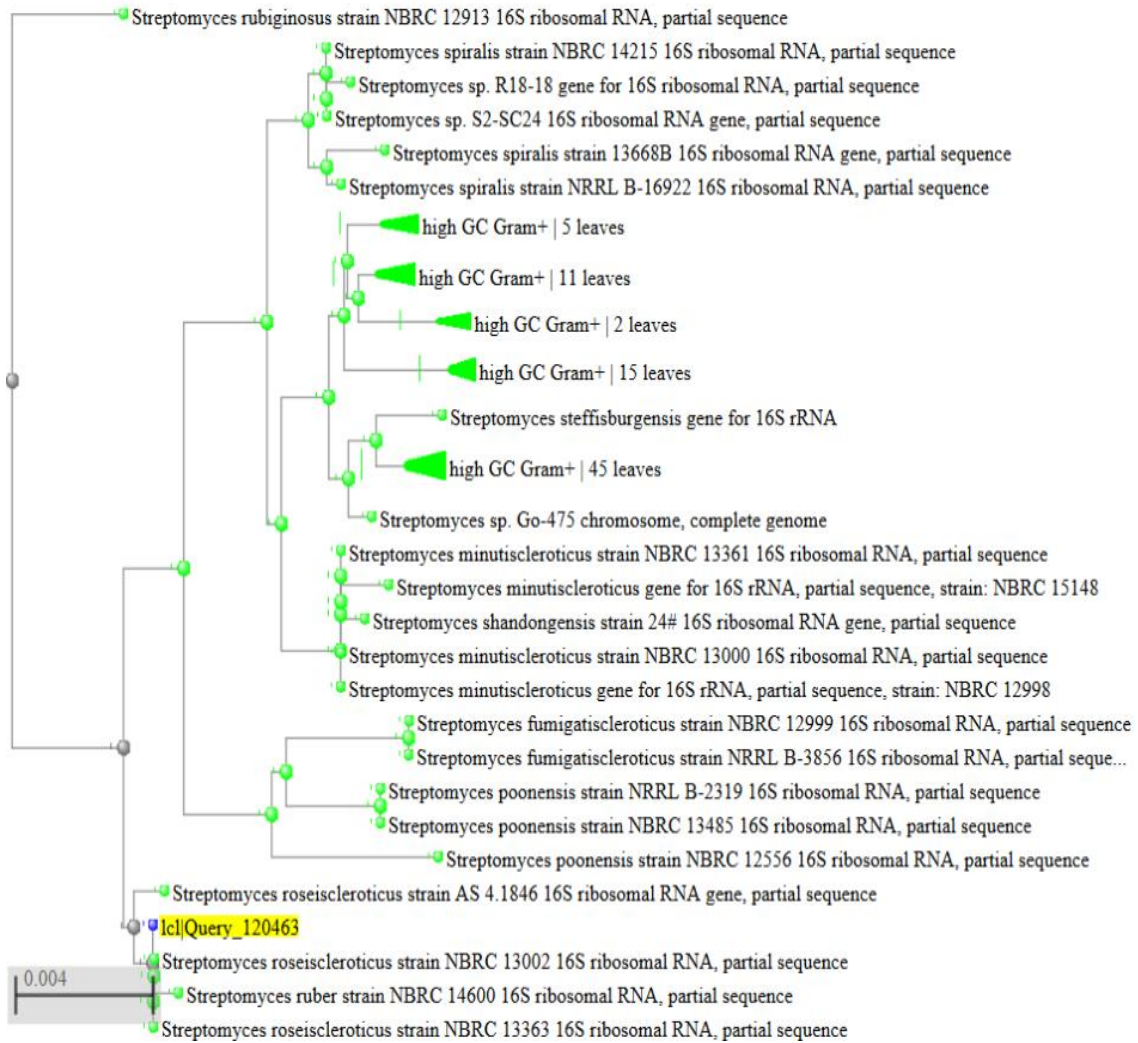


Fig. 2. Phylogenetic analysis of the 16 S rRNA genes from the microbial isolate, evolutionary history was inferred by using the neighbor-joining method. The scale bar represents 0.004 substitutions per base position

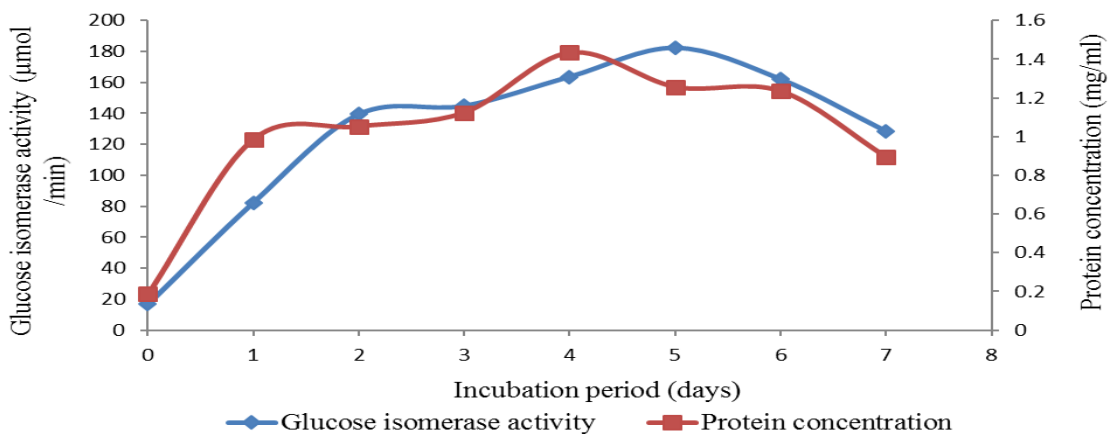


Fig. 3. Glucose isomerase activity on various incubation days (day 0 to day 7) using 0.1M glucose as substrate, 0.1 M phosphate buffer pH 7.0 and 0.05 M MgSO₄. Peak glucose isomerase activity was obtained after 120 hr (five days)

Table 3. NCBI blast result for the query sequence

S/No	Sample name	NCBI identification Species name	Total Score	Query Cover	E value	Similarity (%)	Accession number
1		<i>Streptomyces roseiscleroticus</i>	2614	100	0.0	99.51	NR 112381

Bootstrap score often shows the levels of relatedness or closeness between the query sequence and other homologous sequences from the NCBI Genbank. A bootstrap score of 99.51 % is indicative of a strong relatedness between the query sequence and *Streptomyces roseiscleroticus* strain AS 4.186.

Studies on the glucose isomerase production using *Streptomyces roseiscleroticus* showed that maximum glucose isomerase production was achieved on day five of the submerged fermentation (Fig. 3).

There was a gradual increase in the intracellular glucose isomerase production from day one to day five, beyond which, glucose isomerase production decreased. The gradual increase in glucose isomerase production could possibly be attributed to the fact that *Streptomyces* are slow growers in their fermentation broth [13] as they require some time to acclimatize in their growth or fermentation medium. Also, maximum glucose isomerase production on day five could be indicative of the logarithmic phase or the exponential phase of microbial growth often characterized by nutrient availability and massive microbial cell doubling. Furthermore, the decrease in glucose isomerase production from day five through day seven could be suggestive of the microorganism attaining its stationary phase of microbial growth or the depletion of the nutrients of the microbial broth. An incubation period of five days obtained in this study agrees with the work of Yassien and Jiman [6] who reported an incubation period of 120 hr for glucose isomerase production from *Streptomyces albaduncus*. Similarly, Pandidurai et al [2] reported an incubation period of 36 hr for optimum glucose isomerase production from *Enterobacter agglomerans*. Variations in incubation periods for glucose isomerase production could be attributed to the differences in the type and strain of the microorganism used. Similarly, the protein concentration of the various fermentation period was also monitored. The highest protein concentration was obtained on day four (96 hr). This is very likely to be attributed to the protein compositions (beef extract, yeast extract and peptone) of the

fermentation broth absorbed by the microorganism.

4. CONCLUSION

The microbial strain, *Streptomyces roseiscleroticus* showed good glucose isomerase producing capacity. Findings from this study hence suggests that *Streptomyces roseiscleroticus* can serve as a potential source of glucose isomerase production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Habeeb S, Yazaji S, Al-Amir L. Optimization of glucose isomerase production from *Streptomyces* sp. SH10 using the response surface methodology. International Food Research Journal. 2016;23(2):756-761.
- Pandidurai RNS, Kalaihelvan PT, Kumar DJM, Gnanaraj M. Production of extracellular glucose isomerase by *Enterobacter agglomerans* isolated from garden soil. International Journal of Current Research. 2011;3:21 -25
- Faiz O, Ahmet C, Yakup K, Nagihan SE. Cloning expression and characterization of xylose isomerase from thermophilic *Geobacillus caldoxylosilyticus* TK4 strain. Turkish Journal of Biochemistry. 2011;36 (1):6–14.
- Bhosale SH, Rao MB, Deshpande VV. Molecular and Industrial Aspects of Glucose Isomerase. Microbiological Reviews. 1996;60:280–300.
- Fenn TD, Dagmar R, Petsko GA. Xylose isomerase in substrate and inhibitor michaelis states: atomic resolution studies of a metal mediated hydride shift. Biochemistry. 2004;43:6464-6474.
- Yassien MAM, Jiman FAA. Optimization of glucose isomerase production by *Streptomyces albaduncus*. African Journal

- of Microbiology Research. 2012;6(12): 2976-2984.
7. Alessandra B, Simona S. Industrial applications of immobilized enzymes - A review. *Molecular Catalysis*, 2019;479:1-20.
 8. Yassien M, Jiman F. Purification, characterization and immobilization of glucose isomerase from *Streptomyces albaduncus*. *African Journal of Microbiology Research*. 2013;7(21):2682-2688.
 9. Ramagopal UA, Dauter M, Dauter Z. Phasing on anomalous signal of sulfurs: what is the limit? *Acta Crystallographica D-Biological Crystallography*. 2003;59(6): 1020–1027.
 10. Bhasin S, Sharma P, Rajpai P, Modi HA. Comparative Study of Extraction Methods for Intracellularly Produced Glucose Isomerase by *Streptomyces sp. SB – AII4*. *International Research Journal of Biological Sciences*. 2013;2(10):43-50.
 11. Martin JF, Aparicio JF. Enzymology of the polyenes pimaricin and candicidin biosynthesis. *Methods in Enzymology*. 2009;459:215-242.
 12. Muhammad I, Muhammad JA, Muhammad G, Nazia M, Nyla J, Saqib HH, Irfan M, Zahid A, Dawood Ahmed. Hyper production of glucoamylase by *Aspergillus niger* through the process of chemical mutagenesis. *International Journal of the Physical Sciences*. 2011;6:6179-6190.
 13. Hasani A, Kariminik A, Issazadeh K. Streptomycetes: Characteristics and Their Antimicrobial Activities. *International Journal of Advanced Biological and Biomedical Research*. 2014;2:63-75.
 14. Kariminik A, Baniyasi F. Pageant agonistic activity of actinomycetes on some gram negative and gram positive bacteria. *World Applied Sciences Journal*. 2010;8(7):828-832.
 15. Berdy J. Bioactive microbial metabolites: a personal view. *Journal of Antibiotics*. 2005;58(1):126.
 16. Bibb M. Regulation of secondary metabolism in *Streptomyces*. *Current opinion in microbiology*, 2005;8:208-215.
 17. Mann J. Natural products as immunosuppressive agents. *Natural Product Reports*. 2001;18:417-430
 18. Givry S, Duchiron F. Optimization of culture medium and growth conditions for production of L-arabinose isomerase and D-xylose isomerase by *Lactobacillus biferrmentans*. *Microbiology*. 2007;77: 281-287.
 19. Dong-Xu J, Lin Z, Yu-Guo Z. Properties of a novel thermostable glucose isomerase mined from thermus oshimai and its application to preparation of high fructose corn syrup. *Enzyme and Microbial Technology*. 2017;99:1–8.
 20. Silva RDN, Quintino FP, Monteiro VN, Asquieri ER. Production of glucose and fructose syrups from cassava (*Manihotesculenta Crantz*) starch using enzymes produced by microorganisms isolated from Brazilian Cerrado soil. *Food Science and Technology*. 2010;30(1):213-217.
 21. Robert SB, Murray EG, Nathan RS. Berger's manual of determinative bacteriology (7th Edition). Baltimore, USA. 1957;632-635.
 22. Sapunova LI, Labanok AG, Kazakevich IO, Evtushenkov AN. A plate method to screen microorganisms producing Xylose Isomerase. *Microbiology*. 2004;73: 107:112.
 23. Dische Z, Borenfreund E. A new spectrophotometric method for the detection and determination of keto sugars and trioses. *The Journal of Biological Chemistry*. 1951;192(2):583-587.
 24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*. 1951;193(1):265-275.

© 2021 Chinyelum et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
 The peer review history for this paper can be accessed here:
<https://www.sdiarticle4.com/review-history/72684>