







ORIGINAL RESEARCH ARTICLE

Endophytic and Rhizospheric Actinobacteria from *Guiera senegalensis* as Novel Sources of Glucose Isomerase under Submerged Fermentation

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ABSTRACT

Glucose isomerase (GI) is a key industrial enzyme used to convert glucose to fructose in the production of high-fructose corn syrup (HFCS). Despite the widespread application of GI, there is a growing need to explore novel and cost-effective microbial sources, particularly endophytic Actinobacteria, which are known for their metabolic versatility and enzyme-producing capabilities. However, there is no evidence of GI production potential from Actinobacteria associated with *Guiera senegalensis*. This study aimed to isolate endophytic and rhizospheric Actinobacteria from *Guiera senegalensis* and evaluate their potential for Glucose isomerase production under submerged fermentation conditions. Endophytic Actinobacteria were isolated from the roots of *G. senegalensis* obtained from farmland within Abdullahi Fodio University of Science and Technology, Aliero (AFUSTA). GI production was performed via submerged fermentation for 72 hours, with enzymatic activity assessed at 24-hour intervals. The six (6) isolated Actinobacterial strains appeared in different colours (white, yellow, and pale yellow) and textures (filamentous, moist, convex, and fluffy). GI activity was measured by measuring the absorbance of the 2 mL reaction mixture in triplicate. Fructose concentration was determined from a calibration curve prepared using standard fructose solutions (0–100 µg/mL). One unit (U) of glucose isomerase activity was defined as the amount of enzyme required to catalyze the formation of 1 µmol of fructose per minute under the specified assay conditions. The GI activity produced by six (6) isolates was determined to be 5.22±1.03 EU/ml, 4.85±0.54 EU/ml, 5.07±0.33 EU/ml, 5.0±0.78 EU/ml, 4.28±0.65 EU/ml, and 4.92±0.48 EU/ml. Isolate S-1 recorded the highest activity (5.22±1.03EU/ml). The optimal temperature and pH for GI production were determined to be 35 °C and 6, respectively. Kinetic parameters (Km) and Vmax were found to be 2.3202 mM and 39.0625 U/mL, respectively. These findings demonstrate that endophytic Actinobacteria associated with *G. senegalensis* represent a promising microbial resource for GI production and High Fructose Corn Syrup production, an essential sweetener in the food and beverage industries. Further studies on strain improvement and large-scale production are recommended to enhance its industrial production.

INTRODUCTION

Enzymes are biological catalysts that play a crucial role in various industrial applications, particularly in food, pharmaceutical, and biofuel industries. Their ability to accelerate biochemical reactions under mild conditions has revolutionized food processing, improving efficiency and product quality. Enzymes are widely used in dairy, brewing, meat processing, baking, juice and beverage production, vegetable processing, dietary supplements, and the oil and fat industries (Singh *et al.*, 2020). Historically, the use of enzymes and microorganisms in

food processing dates back centuries, with applications in bread baking, beer brewing, and cheese-making. Modern biotechnology has further advanced the use of enzymes, offering novel approaches to enhance the conversion of raw materials into high-value food products (Kognou *et al.*, 2023).

One approach derived from modern biotechnology is the use of Glucose isomerase to produce High fructose corn syrup (HFCS), a sweetener that needs to replace the

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existing chemical sweeteners. HFCS has numerous benefits, including a low calorific value, non-insulin-dependent absorption, and easy transportation in its liquid form. Unlike HFCS, the existing sweeteners are not suitable for diabetic patients; therefore, the food and beverage industries need to replace them with healthier alternatives (Singh *et al.*, 2020).

Glucose isomerase (GI), also known as xylose isomerase, is an industrially significant enzyme that catalyzes the reversible isomerization of D-glucose to D-fructose. This enzyme is widely used in the production of high-fructose corn syrup (HFCS), a key sweetener in the food industry. GI is among the top three industrial enzymes, alongside amylases and proteases, due to its widespread applications in the baking, canning, pharmaceutical, and biofuel sectors (Saikia *et al.*, 2022). The commercial production of HFCS involves enzymatic isomerization, offering advantages such as high specificity, low energy consumption, minimal by-product formation, and superior taste profiles compared to chemical methods (Singh *et al.*, 2020). HFCS formulations vary based on fructose content, including HFCS 42 (42% fructose, 58% glucose), HFCS 55 (55% fructose, 45% glucose), and HFCS 90 (90% fructose, 10% glucose) (Al-Dhabi *et al.*, 2020).

The production of glucose isomerase has traditionally relied on microbial sources, including bacteria and fungi, due to their rapid growth rates and ease of genetic manipulation. Bacterial sources, particularly *Streptomyces*, *Bacillus*, *Actinoplanes*, and *Arthrobacter*, have demonstrated high GI yields under optimized conditions (Singh *et al.*, 2020). Several factors, including carbon and nitrogen sources, pH, and temperature, inducers, aeration, and substrate particle size, influence the selection of microbial strains for GI production. Soil ecosystems serve as a reservoir of diverse microbial species with potential industrial applications. Bacteria isolated from extreme environments, such as arid soils, often exhibit enhanced enzyme stability and activity, making them promising candidates for industrial enzyme production. Lignocellulosic biomass and agricultural waste have emerged as sustainable feedstocks for GI production, thereby reducing production costs and mitigating environmental impact (Acharyabhata *et al.*, 2013). The enzymatic conversion of these substrates offers an eco-friendly alternative to traditional chemical processes, aligning with global efforts to develop sustainable biotechnological solutions. Several studies have reported the successful production of GI using agricultural residues as substrates, highlighting their potential for large-scale bioprocessing (Rengasamy *et al.*, 2020).

Despite significant advancements in GI production, there remains a need to explore novel microbial sources capable of withstanding high temperatures in industrial GI production. *Guiera senegalensis*, a medicinal plant widely distributed across West Africa, is known to withstand drought and survive high temperatures in desert regions during the dry season. Therefore, root and soil samples

associated with this plant may contain bacteria capable of producing industrially relevant enzymes (including GI) even at higher temperatures.

Recently, various studies have isolated numerous bacteria with the capability of producing glucose isomerase, including *Bacillus*, *Paenarthrobacter*, and *Mycobacterium*, from soil samples. Optimal GI production was achieved under submerged fermentation at 40°C, pH 6–8, with specific nutrient combinations (peptone/yeast extract or tryptone/peptone) and carbon sources (xylose, wheat straw). These findings from various studies support the feasibility of using plant-associated actinobacteria for GI production, but direct evidence from *Guiera senegalensis* is lacking (Iftikhar *et al.*, 2019). Therefore, the present study aims to isolate glucose isomerase-producing bacteria from soil samples collected from *Guiera senegalensis* plants at Sokoto State University. The findings from this study could contribute to the development of cost-effective and sustainable strategies for industrial GI production.

MATERIALS AND METHODS

Reagents used

All chemicals and reagents used in this study were of analytical grade and obtained from Inqaba Biotec (South Africa) and Hali Shuaibu Chemicals, Sokoto (Nigeria). Sterile distilled water and microbiological media were prepared according to standard laboratory protocols.

Sample Collection and Isolation of Actinobacteria

Rhizospheric soil samples were manually screened to remove unwanted materials such as gravel, plant debris, and other foreign particles. A 1 g soil sample was suspended in 9 mL of sterile distilled water in a test tube and vortexed for 1 minute to achieve uniform dispersion. The suspension was serially diluted tenfold from 10^{-1} to 10^{-5} . From the 10^{-3} and 10^{-5} dilutions, 100 μ L was spread-plated onto Starch Casein Agar (SCA) medium using a sterile glass spreader. The plates were labelled S-1, S-2, and S-3. For root-associated Actinobacterial isolation, root samples were washed thoroughly with running tap water to remove adhering soil particles, then surface-sterilized with 70% ethanol for 30 seconds and rinsed in sterile distilled water. The roots were then mechanically homogenized using a sterile mortar and pestle, and a small portion was suspended in 9 mL of sterile distilled water. The homogenate was serially diluted tenfold from 10^{-1} to 10^{-5} . From the 10^{-2} and 10^{-3} dilutions, 100 μ L was spread-plated onto SCA medium. The plates were labelled R-1, R-2, and R-3. All plates were incubated at 30°C for 3–7 days under aerobic conditions. Distinct colonies with Actinobacterial features were carefully picked using a sterile inoculating loop and streaked onto freshly prepared SCA plates. The plates were incubated at 37°C for 4 days to allow sufficient growth of the subcultured isolates. Pure cultures were maintained at 4°C on SCA slants for further study.

Glucose Isomerase Production

The production medium was prepared using the following components (per 500 mL of distilled water): 5 g rice husk, 0.7 g peptone, 0.75 g yeast extract, 1.5 g KH_2PO_4 , 3 g K_2HPO_4 , 1 g KCl, 0.5 g KNO_3 , 0.25 g MgCl_2 , 1.5 g CaCO_3 , and 0.01 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The medium was heated to boiling to ensure complete dissolution of the components. Sterilization was performed by autoclaving at 121°C and 15 psi for 15 minutes, after which the medium was cooled to approximately 50°C. To prevent fungal and Gram-negative bacterial contamination, cycloheximide (50 µg/mL) and nalidixic acid (50 µg/mL) were added and mixed thoroughly. A starter culture was prepared by inoculating individual bacterial colonies from subcultured plates into 50 mL of the production medium contained in a 200 mL conical flask. The inoculated medium was incubated in a shaking incubator at 40°C for 24 hours to allow for bacterial growth and adaptation. Following this, 50 mL of the starter culture was transferred into a fresh 450 mL production medium in a 1 L Erlenmeyer flask and incubated under shaking conditions (180 rpm) at 40°C for 72 hours to facilitate enzyme production. A negative control was prepared by maintaining an identical 500 mL production medium without bacterial inoculation to account for any background enzymatic activity originating from the medium components. Following 72 hours of fermentation, the culture was centrifuged at $10,000 \times g$ for 10 minutes at 4°C to separate the bacterial cells from the extracellular enzyme. The supernatant was carefully collected and stored as the crude extracellular glucose isomerase enzyme for further analysis.

Glucose isomerase Activity test

Glucose isomerase (GI) activity was assessed using a spectrophotometric method based on the quantification of fructose formation. The reaction mixture (total volume: 2.0 mL) consisted of 500 µL of 0.5 M phosphate buffer (pH 7.5), 200 µL of 1 M glucose, 100 µL of 0.1 M MgSO_4 , 100 µL of 0.01 M CoCl_2 , and 200 µL of crude enzyme extract. The reaction was incubated at 70°C for 1 hour in a thermostatic water bath. A control reaction containing heat-denatured enzyme was prepared under identical conditions. The reaction was terminated by adding 2.0 mL of 0.5 M perchloric acid, followed by centrifugation at $10,000 \times g$ for 10 minutes at 4°C to remove precipitated proteins. To estimate the fructose concentration, 50 µL of the supernatant was diluted with 950 µL of distilled water (dH_2O), followed by the sequential addition of 200 µL of 1.5% (w/v) cysteine hydrochloride, 6.0 mL of 70% (v/v) sulfuric acid (H_2SO_4), and 200 µL of 0.12% (w/v) alcoholic carbazole solution. The reaction mixture was vortexed and incubated at 30°C for 30 minutes for colour development. The absorbance of the resultant purple chromophore was measured at 560 nm using a UV-visible spectrophotometer against a reagent blank. Fructose concentration was determined from a calibration curve prepared using standard fructose solutions (0–100 µg/mL). One unit (U) of glucose isomerase activity was defined as the amount of enzyme required to catalyze the

formation of 1 µmol of fructose per minute under the specified assay conditions. All measurements were performed in triplicate, and results were expressed as mean \pm standard deviation (SD).

Optimization of fermentation conditions

The effect of temperature and pH for the Glucose Isomerase production was optimized by setting the incubator at various temperatures (25°C, 30°C, 35°C, 45°C and 50°C) and varying pH of 3, 4, 5, 6, 7, 8 and 9 in the production media.

Kinetic behaviour of GI

Kinetic properties of GI were determined using the Lineweaver–Burk plot. Glucose was used as the substrate for studying the kinetic properties of GI. The substrate used in this study was introduced at concentrations ranging from 0.1 to 2.0 M. The pH for GI kinetic studies was 7.0, and the temperature was kept at 70°C.

RESULTS

Morphologically distinct colonies of *Actinobacteria* isolated from rhizospheric soil and roots differ in size, shape, and colour; they include small and large white colonies with powdery sporulation and yellow and pale yellow colonies with pigment diffusing through the media. (Figure 1, Table 1). Pure isolates of both endophytic and rhizospheric *Actinobacteria* were obtained through subculture (Figure 2). Glucose isomerase activity from the six (6) isolated *Actinobacterial* strains (Figure 4, Table 2).

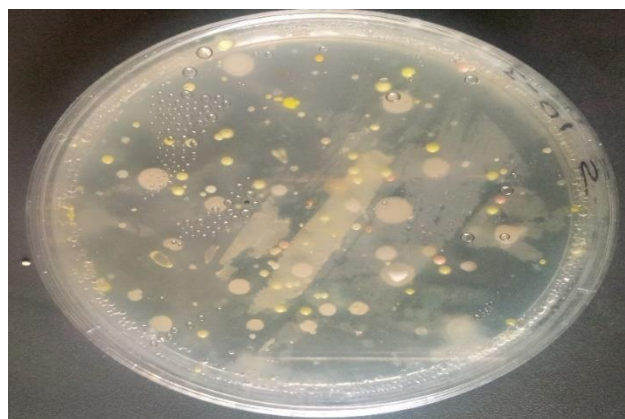


Figure 1: isolated endophytic *Actinobacterial* strains

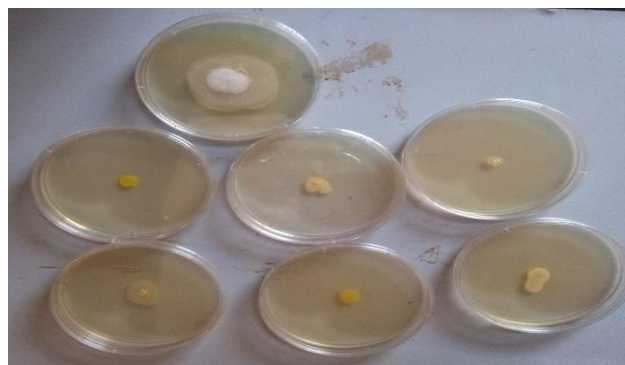


Figure 2: Subcultured *Actinobacterial* strains from both root and rhizospheric soil of *Guiera senegalensis*.

Table 1: Isolation and Morphological Characterization of Actinobacteria

Morphotype	Colony Characteristics	Sporulation	Pigmentation
Group I	Small, powdery, white colonies	Dense	Non-diffusible
Group II	Large, powdery, white colonies	High	Non-diffusible
Group III	Yellow colonies with raised structure	Moderate	Diffusible
Group IV	Light pink colonies, compact	Low	Diffusible

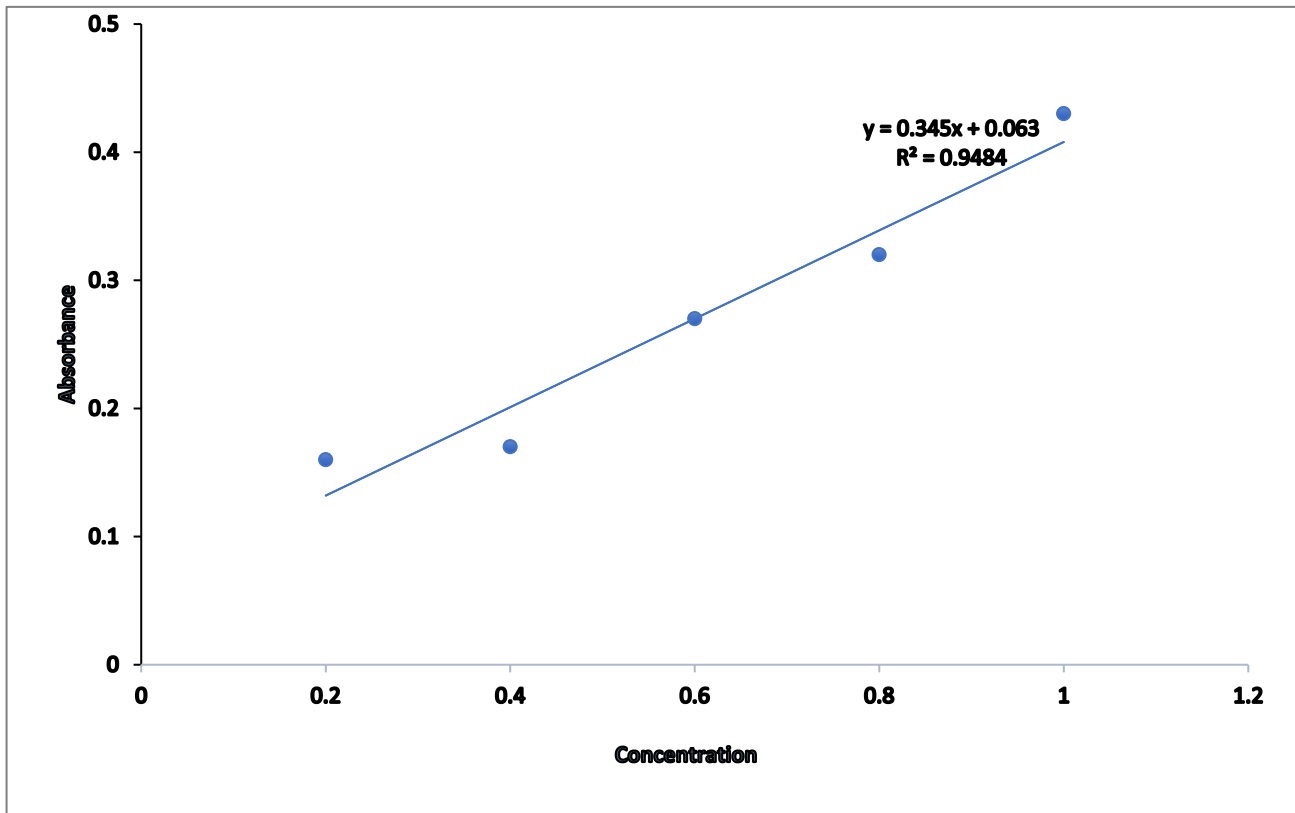


Figure 3: Fructose calibration curve

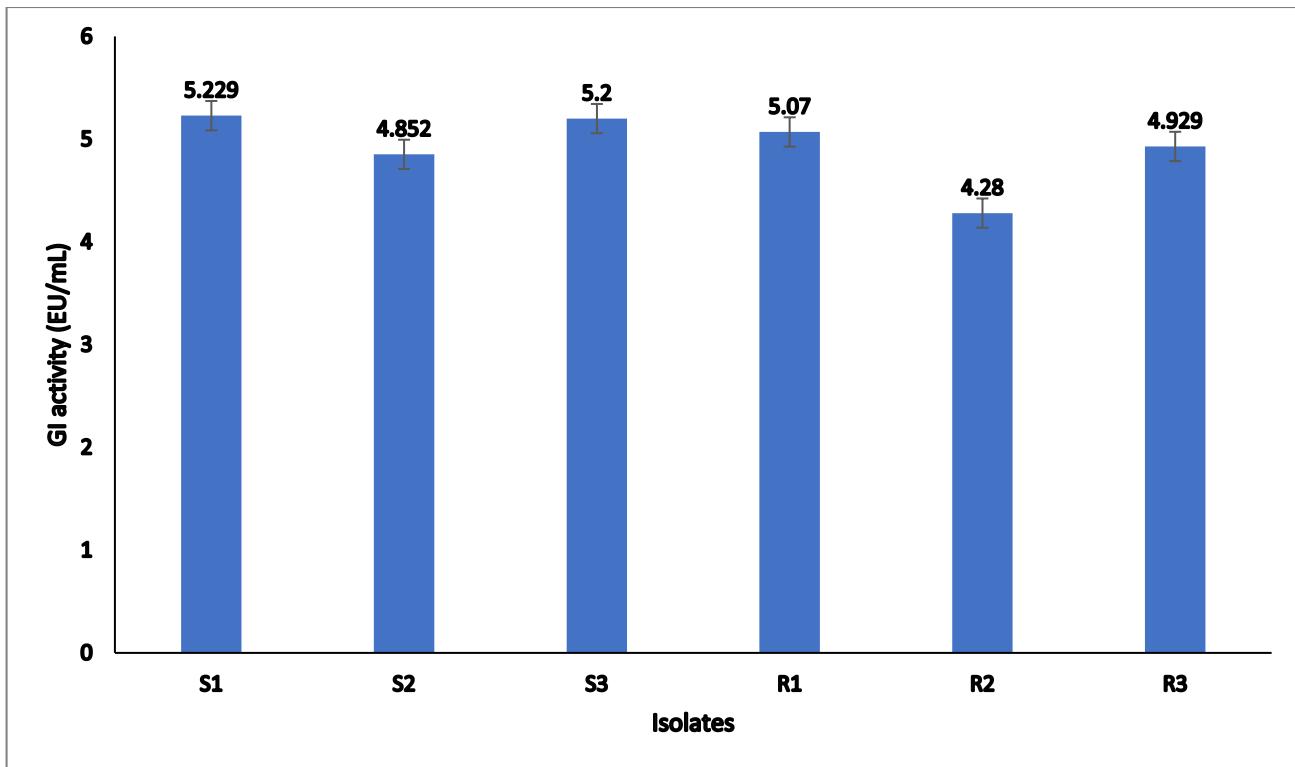


Figure 4: Glucose isomerase activity of the six isolated strains from the root and rhizospheric soil

Key: S1-S3 = Bacterial strains isolated from the rhizospheric soil sample

R1-R3 = Bacterial strains isolated from the root sample

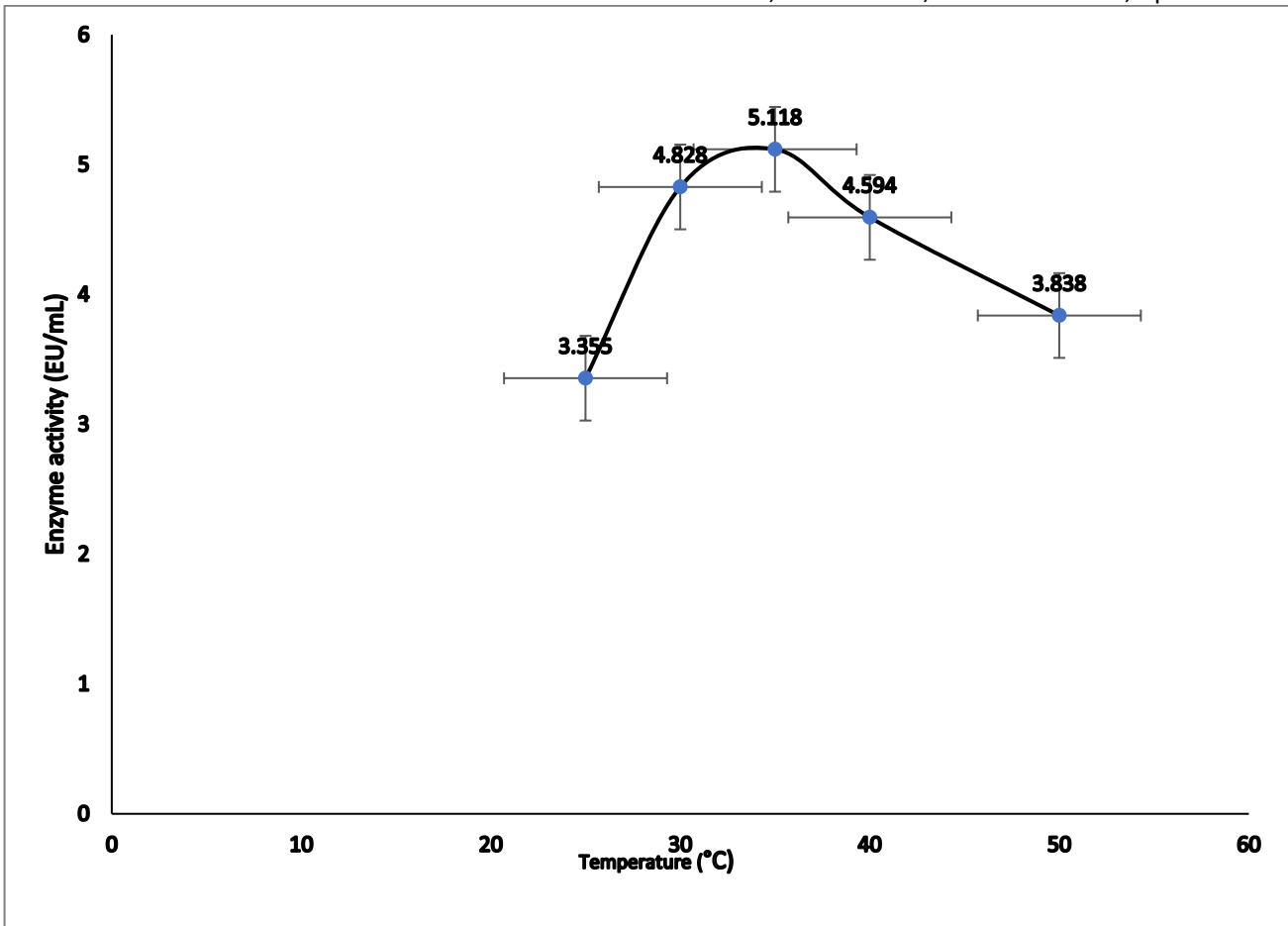


Figure 5: Effect of incubation temperature on GI yield

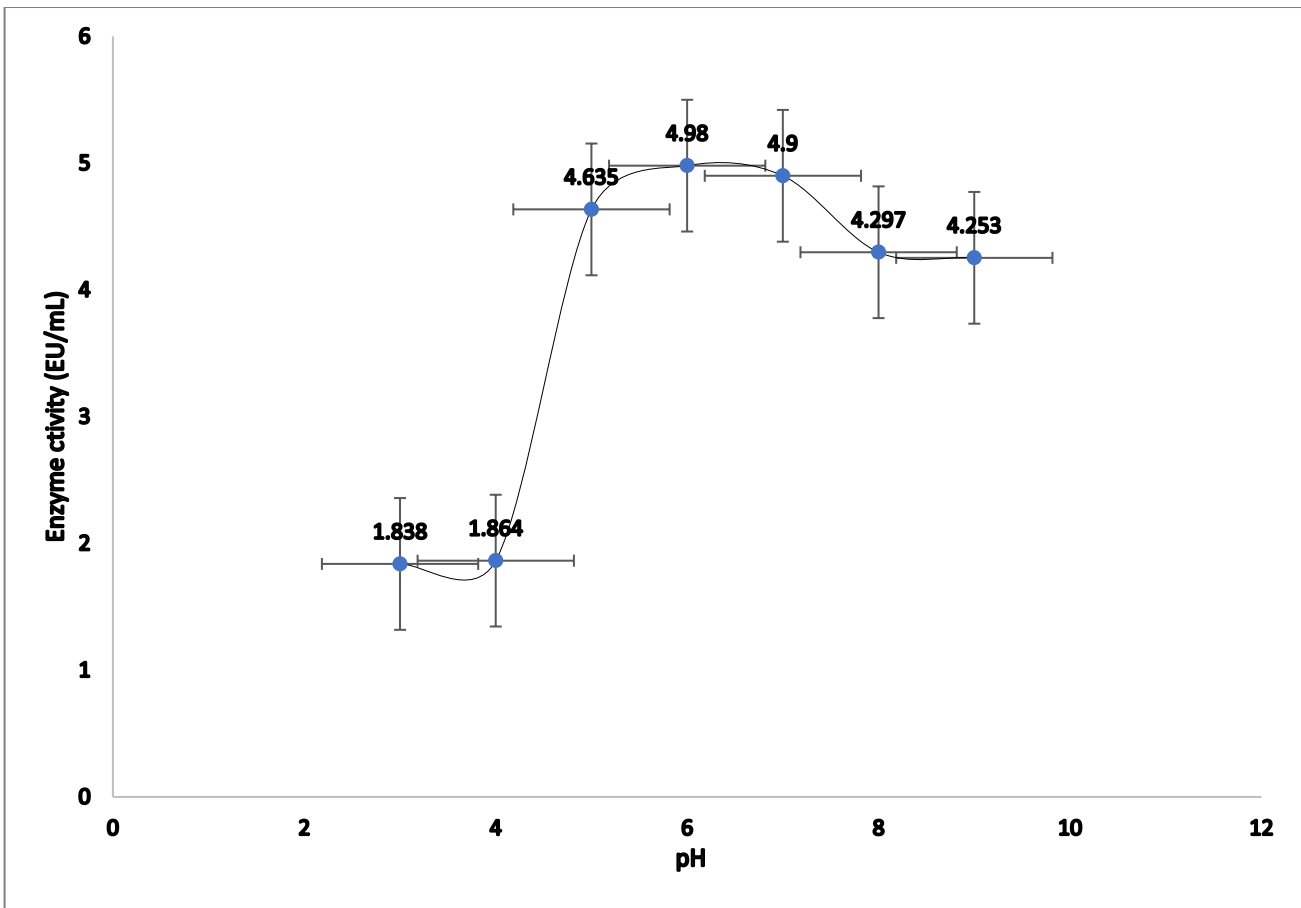


Figure 6: Effect of pH on GI yield

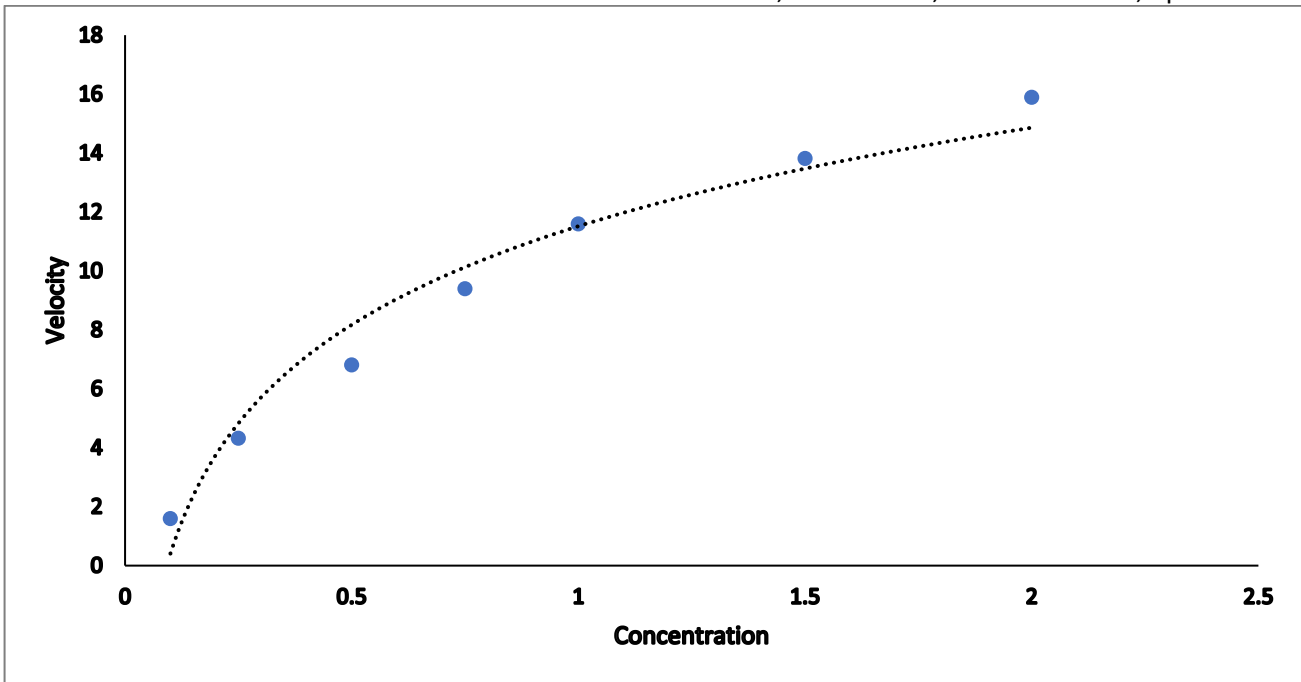


Figure 7: Michaelis-Menten plot of GI activity from S-1

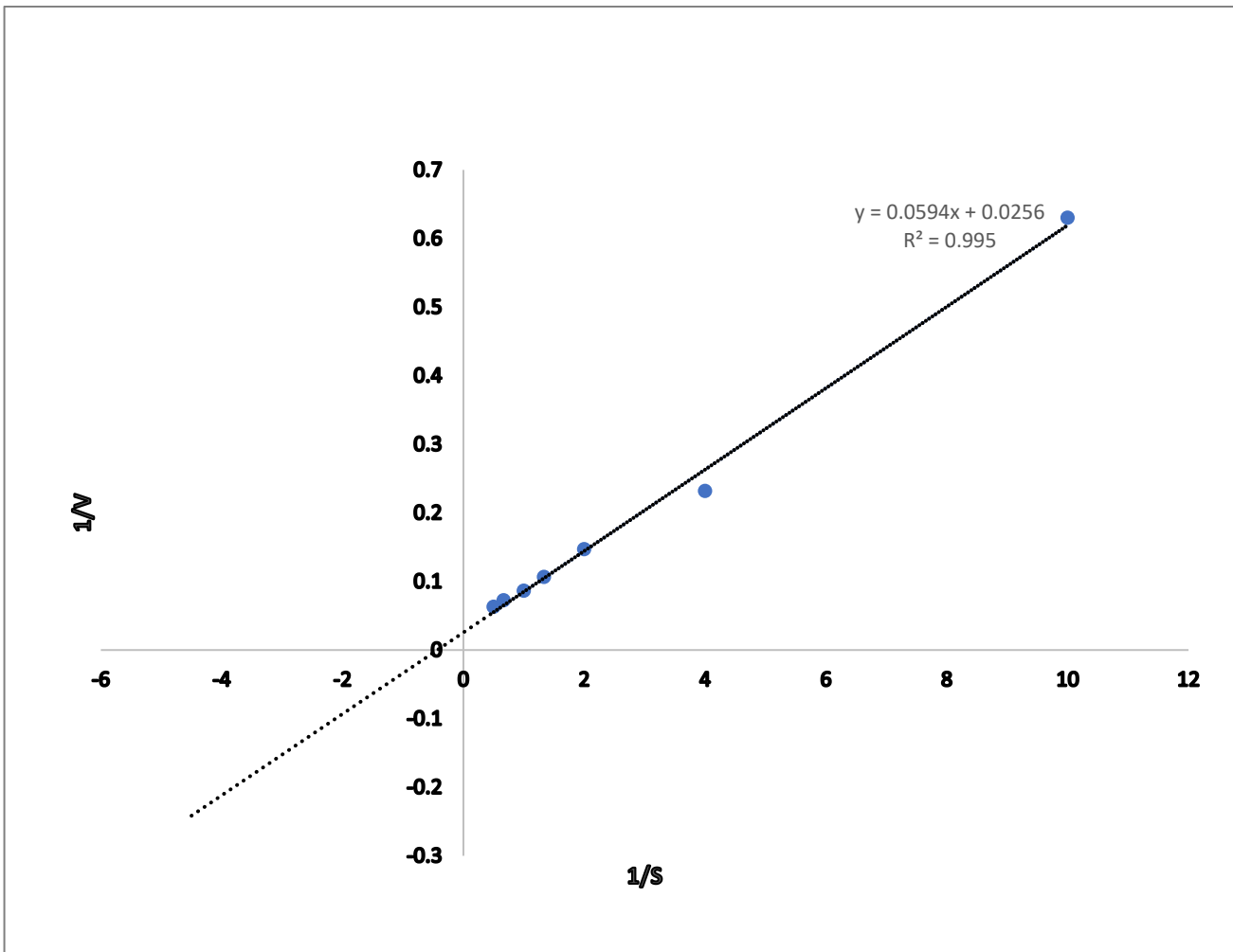


Figure 8: Line weaver burk plot for GI activity from S-1

Optimization of Fermentation Conditions (Temperature and pH)

The optimum temperature for glucose isomerase production was found to be 35°C. The impact of

incubation temperature (25–50°C) on GI enzyme yield was assessed. Enzyme production increased significantly up to 35°C, beyond which activity declined due to thermal denaturation (Figure 5, Table 3). The optimum pH for the GI production was determined to be 6. Enzyme

production was evaluated across a pH range of 3.0–9.0. Maximum yield was recorded at pH 6.0 (Figure 6, Table 4).

Table 2: Enzyme (GI) assay of the six isolates

Isolate	Enzyme Activity (EU/ml)	SD
S-1	5.229	± 1.03
S-2	4.852	± 0.54
S-3	5.2	± 0.33
R-1	5.07	± 0.78
R-2	4.28	± 0.65
R-3	4.929	± 0.48

The values refer to enzyme activity in EU/ml (mean ± SD, n=3)

Table 3: Effect of temperature on GI activity

Temperature (°C)	GI Activity (EU/ml)	SD
25	3.355	± 0.64
30	4.828	± 0.74
35	5.118	± 0.57
40	4.594	± 0.34
50	3.838	± 0.45

A one-way ANOVA test confirmed a significant difference in enzyme activity across temperature ranges ($p < 0.05$), indicating that GI functions optimally at 35°C, but this is the production, not activity, temperature.

Table 4: GI activity at different pH levels

pH	GI Activity (EU/ml)	Standard Deviation
3	1.838	± 0.68
4	1.864	± 0.33
5	4.635	± 0.53
6	4.98	± 0.84
7	4.9	± 0.49
8	4.297	± 0.46
9	4.253	± 0.64

A t-test comparing GI activity at pH 7.0 and pH 6.0 showed significant variation ($p < 0.01$), confirming optimal conditions for GI expression.

Kinetic behavior of GI

The Michaelis–Menten constant (K_m) for GI was found to be 2.3203mMol. The Lineweaver–Burk plot showed that the maximum velocity (V_{max}) for GI is 39.0625 U/ml, (Figures 7 and 8).

DISCUSSION

The present study focused on the production and optimization of glucose isomerase (GI) in submerged fermentation using an isolated bacterial strain. The results were analyzed in light of the literature, providing insights into the enzyme's optimal production conditions and kinetic parameters.

Morphologically distinct colonies of *Actinobacteria* were isolated from both root and rhizospheric soil of *Guiera senegalensis* on starch-casein agar medium. The colonies showed variation in size, shape, and pigmentation. They include small and large white colonies with powdery sporulation, and yellow and light pink colonies with pigment diffusing through the media (Bahauddeen and Almajir, 2020; Salisu et al. 2019, 2020, 2022) This diversity

is consistent with an earlier report on *Actinobacteria* (Singh et al., 2020), highlighting the metabolic versatility of this group. Pure isolates from both endophytic and rhizospheric *Actinobacteria* produced glucose isomerase (GI) with activities ranging from 4.39 to 5.29 EU/ml. These values are comparable to those reported by (Singh et al., 2020) on six different isolates, ranging from (1.3 – 4.27 EU/ml) produced under similar submerged fermentation conditions. This finding suggest that *Guiera senegalensis*-associated *Actinobacteria* represent a competitive microbial source for GI production.

Temperature plays a crucial role in microbial enzyme production by influencing metabolic activity, enzyme stability, and substrate-enzyme interactions. The optimum temperature for GI production in this study was determined to be 35°C, while the activity assay was performed at 70°C according to standard GI protocols. This finding is consistent with the report by Gomez et al. (2021), who demonstrated that GI production in submerged fermentation achieves maximum efficiency at a similar temperature. However, Kognou et al. (2023) reported that the optimal temperature for GI production in *Bacillus sp.*, *Paenarthrobacter sp.*, *Chryseobacterium sp.*, *Hymenobacter sp.*, *Mycobacterium sp.*, and *Stenotrophomonas sp.* was 40°C. Specifically, *Stenotrophomonas sp.* exhibited the highest isomerization activity at this temperature, suggesting that species-specific variations exist in GI thermal stability and enzyme kinetics.

The discrepancy between our findings and those of Kognou et al. (2023) could be attributed to differences in bacterial strain metabolism, enzyme structure, and fermentation conditions. While some GI-producing bacterial strains exhibit enhanced enzymatic activity at elevated temperatures (above 40°C), others show peak activity at moderate temperatures due to structural differences in their enzyme active sites and folding stability. This suggests that further investigations, including molecular dynamics simulations and site-directed mutagenesis, could provide deeper insights into the thermal adaptability of GI.

The pH of the fermentation medium is a critical factor affecting enzyme production, activity, and stability. In this study, the optimum pH for GI production was determined to be 6.0, a value that aligns with reports by López-Trujillo et al. (2023) and Rengasamy et al. (2020) and closely corresponds with the findings of Kognou et al. (2022). However, Singh et al. (2020) reported that the optimal pH for GI production by *Bacillus sp.* and *Paenarthrobacter sp.* was 8.0, suggesting that some bacterial strains exhibit higher GI stability under alkaline conditions.

Furthermore, Messaoudi et al. (2020) reported that GI from *Actinoplanes missouriensis* exhibited maximum activity at pH 7.0. Interestingly, our findings also indicate that the GI from isolate S-1 exhibited peak activity at pH 6.0, similar to *Actinoplanes missouriensis*. This reinforces the idea that GI activity remains stable across a pH range of 6.0 to 8.0, making it adaptable for industrial applications where minor pH variations occur during enzymatic reactions.

Variations in optimal pH across studies can be attributed to differences in microbial strains, culture conditions, and enzyme purification methods. Since pH affects the ionization state of amino acid residues at the enzyme's active site, minor structural differences in GI among bacterial species may explain variations in pH stability. Further studies involving protein crystallography and computational modelling could provide insights into the pH-dependent conformational changes in GI.

The kinetic parameters of GI, including the Michaelis-Menten constant (K_m) and maximum reaction velocity (V_{max}), provide valuable insights into enzyme-substrate affinity and catalytic efficiency. In this study, the K_m value for GI was found to be 2.3203 mM, while the V_{max} was 39.0625 U/mL. The relatively low K_m value indicates a high affinity of the enzyme for its substrate, which is desirable for industrial applications requiring efficient glucose conversion.

Sharma *et al.* (2021) reported a lower K_m value of 1.54 mol under similar assay conditions, suggesting that GI from their bacterial isolate exhibited an even higher affinity for glucose than the enzyme studied in this research. Differences in K_m values can arise from variations in enzyme structure, active site configuration, and substrate accessibility. Furthermore, Zhao *et al.* (2025) reported K_m and V_{max} values for GI of 11 mM and 25 U/mg, respectively. The significantly lower V_{max} observed in their study may be due to differences in enzyme purity, reaction conditions, or strain-specific enzymatic kinetics.

It is essential to consider that variations in kinetic parameters across studies may stem from differences in enzyme extraction methods, purification protocols, and assay conditions. Enzyme immobilization techniques and genetic modifications aimed at improving substrate-binding efficiency could further enhance GI's industrial applicability.

The use of rice husk in this study as a carbon source represents an environmentally friendly, low-cost approach to enzyme production, aligning with sustainable bioprocessing strategies reported by Acharyabhata *et al.* (2013). However, this study used only crude GI extracts and did not assess GI stability under different temperature and pH conditions, which are essential for industrial applications.

Therefore, further studies should focus on strain improvement, molecular identification of high-yielding isolates, and purification of the GI produced. The use of *Guiera senegalensis* as a source of endophytic *Actinobacteria* also opens an avenue for exploring medicinal plants a reservoirs of industrially relevant enzymes.

CONCLUSION

Endophytic *Actinobacteria* isolated from *Guiera senegalensis* is a suitable option for the industrial production of Glucose isomerase because it produces a reasonable GI activity of 5.29 EU/ml. *Guiera senegalensis* is a cheap agricultural source that can be used in the industrial production of a

health-friendly sweetener (high-fructose corn syrup) through the production of Glucose isomerase. Production of the enzyme from readily available plant sources, such as rice husk, provides a cost-effective means of production, alleviating the high cost of synthetic methods for producing industrially important sweeteners. Despite promising results, variations in optimal conditions reported in previous studies highlight the need for further research on strain-specific enzyme characteristics, molecular structure-function relationships, and industrial process optimization.

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