https://doi.org/10.62587/AFRJBS.2.1.2025.50-60 ISSN: 3006-7464

African Research Journal of Biosciences

Journal homepage: http://www.afrjbs.com

Research Paper Open Access

Enzymatic profiles of *Streptomyces* isolates from Kogi state soil: Biodegradation and environmental applications

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Article Info

Volume 2, Issue 1, January 2025
Received : 30 August 2024
Accepted : 11 December 2024
Published : 25 January 2025
doi: 10.62587/AFRJBS.2.1.2025.50-60

Abstract

This study examines the enzymatic profiles, biochemical characteristics, morphological traits, and molecular identification of ten *Streptomyces* isolates from soil samples in Kogi State, Nigeria, with an emphasis on their potential in biodegradation and environmental biotechnology. Enzymatic assays revealed that isolate S4 exhibited the highest cellulase activity (12 mm zone of clearance), while isolate S3 showed significant ligninase activity (absorbance of 0.85 at 530 nm). Notably, isolate S7 demonstrated pronounced protease activity (15 mm zone of clearance). Biochemical tests revealed diverse metabolic capabilities, with most isolates positive for starch and casein hydrolysis, and five demonstrating gelatin liquefaction. Hydrogen sulfide production was noted in isolates S3 and S4, suggesting their roles in biogeochemical cycling. Morphological analysis indicated considerable diversity in colony color, texture, and shape, aligning with typical *Streptomyces* characteristics. Molecular identification through 16S rRNA sequencing confirmed high similarity (≥99%) to known species such as *Streptomyces coelicolor* and *Streptomyces griseus*. A phylogenetic tree constructed from sequence data illustrated the evolutionary relationships among the isolates. The findings suggest that the *Streptomyces* isolates possess significant enzymatic capabilities, highlighting their potential for biotechnological applications in biodegradation and bioremediation. This research enhances the understanding of *Streptomyces*' ecological roles in soil ecosystems and underscores the need for further exploration of microbial diversity for sustainable environmental management. Future studies should investigate the specific mechanisms underlying the enzymatic activities of these isolates and their practical applications and into optimizing enzyme production and exploring genetic pathways could enhance the practical applications of these isolates, bridging the gap between laboratory findings and real-world implementation.

Key words: *Streptomyces, Enzymatic profiles, Biodegradation, Environmental applications*

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1. Introduction

Streptomyces, a genus within the Actinobacteria phylum, is globally recognized for its extraordinary capacity to produce diverse secondary metabolites and enzymes, many of which are crucial for maintaining soil health

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and ecological equilibrium (Oskay *et al*., 2020). These filamentous bacteria play a pivotal role in the decomposition of organic matter, facilitating nutrient cycling and contributing to soil fertility (Liu *et al*., 2020). Their enzymatic versatility enables them to break down complex organic compounds, including lignin, cellulose, and chitin, making them indispensable agents in natural biodegradation processes and environmental remediation efforts (Dhananjaya *et al*., 2021).

Tropical soils, such as those in Nigeria's Kogi State, represent reservoirs of immense microbial diversity due to their unique climatic conditions, characterized by high temperatures and variable moisture levels. However, these microbial communities remain largely underexplored, limiting our understanding of their ecological roles and potential biotechnological applications (Akinpelu *et al*., 2018). Despite growing global interest in microbial biodiversity, tropical ecosystems are often overlooked, leaving significant gaps in knowledge about their microbial constituents, including *Streptomyces* (Thakur *et al*., 2018).

This study focuses on characterizing *Streptomyces* isolates obtained from soils in Kogi State, Nigeria, with a particular emphasis on their enzymatic capabilities. Enzymes produced by *Streptomyces*, such as cellulases, amylases, proteases, and lipases, are of significant interest due to their potential applications in diverse biotechnological fields, including waste management, bioremediation, and sustainable agriculture. By profiling the enzymatic activities of these isolates, this research seeks to uncover their capacity to degrade various substrates, including environmentally persistent pollutants, thereby illuminating their role in ecological sustainability and their promise as tools for environmental biotechnology.

Moreover, understanding the enzymatic potential of these microorganisms could offer innovative solutions to pressing environmental challenges such as soil contamination, improper waste disposal, and declining agricultural productivity in tropical regions. This investigation not only aims to contribute to the global database of *Streptomyces* diversity but also to explore practical applications that align with sustainable development goals, particularly those focusing on environmental restoration and resource optimization.

2. Materials and methods

2.1. Sample collection and isolation

Soil samples were collected from various sites in Kogi State, Nigeria, using sterile sampling techniques to prevent contamination. The samples were stored in sterilized bags and transported to the laboratory for processing. Isolation of *Streptomyces* was performed using the dilution plate method (Kumar *et al*., 2020). Soil samples were serially diluted, and 100 µL of each dilution was spread onto Starch Casein Agar (SCA) plates supplemented with antibiotics (chloramphenicol) to inhibit the growth of fungi and other bacteria. The plates were incubated at 28°C for 7–14 days, allowing the growth of filamentous actinobacteria. Individual colonies were then picked, sub-cultured, and stored for further analysis.

2.2. Identification of Streptomyces isolates

Morphological characterization of the isolates was conducted based on colony color, texture, shape, and surface appearance (Bérdy, 2005). Biochemical tests for starch hydrolysis, casein hydrolysis, gelatin liquefaction, hydrogen sulfide production, and urease activity were performed following standard microbiological protocols (Cappuccino and Sherman, 2014). The identities of the isolates were confirmed through 16S rRNA gene sequencing. Genomic DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, Germany) following the manufacturer's instructions. PCR amplification of the 16S rRNA gene was conducted using universal primers (27F: 5'-AGAGTTTGATCMTGGCTCAG-3'; 1492R: 5'-TACGGYTACCTTGTTACGACTT-3') (Lane, 1991). The expected product sizes were verified by gel electrophoresis.

2.3. Enzymatic Activity Assays

The enzymatic activities of the *Streptomyces* isolates were assessed through specific assays for cellulase, ligninase, and protease activities.

1. Cellulase Activity: The cellulolytic activity was determined using the Congo Red dye method (Mäkelä *et al*., 2014). Agar plates containing 1% carboxymethyl cellulose (CMC) were inoculated with the isolates and

incubated at 28°C for 7 days. After incubation, the plates were stained with Congo Red solution, and the zones of clearance were measured.

2. Ligninase Activity: Ligninase activity was quantified using a spectrophotometric assay. Isolates were grown in a liquid medium containing lignin as the sole carbon source. After incubation, the supernatant was collected, and absorbance was measured at 530 nm (Oskay *et al.*, 2020).
3. Protease Activity: The proteolytic activity of the isolates was assessed using skim milk agar plates. The plates were inoculated with the isolates and incubated at 28°C for 7 days. Zones of clearance were measured to determine protease activity (Kumar *et al.*, 2020).

2.4. Molecular identification and phylogenetic analysis

The PCR products were purified and sequenced using an ABI 3730 DNA sequencer. The sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) against the NCBI database to determine the closest identified species (Altschul *et al.*, 1990). Phylogenetic relationships were inferred using MEGA X software, employing the Maximum Likelihood method with a bootstrap analysis of 1000 replicates (Kumar *et al.*, 2018).

3. Statistical Analysis

Data obtained from enzymatic activity assays were subjected to statistical analysis using ANOVA to assess significant differences among the isolates. Post-hoc analysis was performed using Tukey's HSD test ($p < 0.05$) to identify specific differences between isolates.

4. Results

4.1. Enzymatic activity

4.1.1. Cellulase activity

All isolates showed cellulolytic activity, with S4 exhibiting the highest zone of clearance (12 mm), followed by S1 (10 mm) and S8 (9 mm) (Table 1).

Table 1: Cellulase activity of *Streptomyces* isolates

Isolate	Zone of clearance (mm)
S1	10
S2	8
S3	7
S4	12
S5	6
S6	5
S7	9
S8	9
S9	6
S10	5

4.1.2. Ligninase activity

Ligninase activity was noted in 7 out of 10 isolates, with S3 showing the highest absorbance (0.85) in the spectrophotometric assay, indicating significant lignin degradation potential (Table 2).

Table 2: Ligninase activity of *Streptomyces* isolates	
Isolate	**Absorbance at 530 nm**
S1	0.60
S2	0.65
S3	0.85
S4	0.70
S5	0.55
S6	0.50
S7	0.75
S8	0.65
S9	0.40
S10	0.30

4.1.3. Protease activity

Protease activity was evident in all isolates, with S7 exhibiting the most significant zone of clearance (15 mm), indicating high proteolytic activity (Table 3).

Table 3: Protease activity of *Streptomyces* isolates	
Isolate	**Zone of clearance (mm)**
S1	10
S2	12
S3	9
S4	11
S5	8
S6	7
S7	15
S8	10
S9	6
S10	5

5. Statistical analysis

ANOVA results indicated significant differences in enzymatic activities among the isolates ($p < 0.05$). Post-hoc analysis confirmed that S4 and S7 had superior enzymatic profiles compared to other isolates.

6. Discussion

This study offers a detailed examination of the enzymatic profiles, biochemical characteristics, morphological traits, and molecular identities of *Streptomyces* isolates obtained from Kogi State soil. These findings enhance our understanding of the functional diversity of *Streptomyces* and underscore their immense potential in various environmental and industrial applications, particularly in biodegradation and biotechnology.

The enzymatic profiles of these isolates reveal their remarkable capacity to degrade complex organic materials, positioning them as promising candidates for environmental biotechnology applications. Among the isolates, S4 stood out for its exceptional cellulase activity, producing a zone of clearance measuring 12 mm (Table 1). This robust cellulolytic activity can likely be attributed to the presence of specific cellulase-encoding genes, enabling efficient hydrolysis of cellulose into simple sugars. Such capabilities are particularly relevant for biofuel production, as cellulose constitutes a major component of agricultural and forestry residues. Efficient cellulose degradation can transform these residues into fermentable sugars, paving the way for sustainable bioethanol production. These findings align with earlier studies that emphasize the cellulolytic potential of *Streptomyces* species in bioenergy research (Mäkelä *et al*., 2014; Oskay *et al*., 2020).

In addition to cellulase activity, isolate S3 exhibited notable ligninase activity, indicated by an absorbance of 0.85 (Table 2). Lignin, a complex aromatic polymer, is notoriously resistant to degradation, making its breakdown a critical challenge in waste management, particularly in industries like paper and pulp processing. The strong ligninase activity observed in S3 suggests its suitability for bioremediation applications targeting lignin-rich waste streams. This enzymatic capability may reflect the adaptation of S3 to lignin-rich environments, where lignin-degrading enzymes play a key role in dismantling lignocellulosic structures. By facilitating the breakdown of lignin, isolate S3 could enhance the availability of simpler organic compounds, contributing to nutrient cycling in soil ecosystems and supporting sustainable waste management practices (Agarwal *et al*., 2019).

Protease activity was another area where the isolates demonstrated substantial enzymatic potential. All isolates exhibited significant proteolytic activity, with isolate S7 emerging as the most proficient, producing a zone of clearance measuring 15 mm (Table 3). Proteases are crucial for breaking down proteins into peptides and amino acids, which are essential for microbial growth and soil nutrient availability. This proteolytic capability also holds significant industrial relevance, particularly in food processing, leather treatment, and waste management industries, where proteases are widely utilized. The pronounced protease activity in isolate S7 suggests its potential for diverse industrial applications and its adaptability to nutrient-rich environments, where efficient protein degradation is vital for microbial survival and competition (Kumar *et al*., 2020).

The observed enzymatic diversity among the *Streptomyces* isolates underscores their functional versatility and adaptability to the environmental conditions of Kogi State soil. This adaptability reflects the evolutionary pressures faced by soil microbes, which necessitate the development of specialized enzymatic systems to exploit various organic substrates. Such enzymatic versatility not only benefits the microbial community itself but also has broader ecological implications, contributing to organic matter decomposition, nutrient recycling, and overall soil health.

Beyond environmental applications, the specific enzymatic capabilities of these isolates open new avenues for their utilization in industrial biotechnology. For instance, the cellulase activity of isolate S4 could be harnessed for biofuel production, while the ligninase activity of isolate S3 offers potential solutions for managing lignin-rich industrial waste. Similarly, the protease activity of isolate S7 can be tapped for applications in food processing and waste treatment industries. The ability to selectively harness these enzymatic activities could lead to innovative and sustainable solutions for pressing environmental and industrial challenges.

The enzymatic profiles of *Streptomyces* isolates from Kogi State soil highlight their functional diversity and significant biotechnological potential. Each isolate exhibited a unique enzymatic strengths that make them suitable candidates for applications in biofuel production, bioremediation, waste management, and industrial enzyme development. These findings not only contribute to our understanding of the ecological roles of *Streptomyces* in tropical soils but also emphasize their value as a resource for addressing global sustainability challenges.

7. Biochemical characteristics

The biochemical characteristics of the *Streptomyces* isolates, as summarized in Table 4, highlight their remarkable metabolic diversity and adaptability to complex soil environments. A majority of the isolates demonstrated

Table 4: Biochemical characteristics of *Streptomyces* isolates

Isolate	Starch hydrolysis	Casein hydrolysis	Gelatin liquefaction	Hydrogen sulfide production	Urease activity
S1	Positive	Positive	Negative	Negative	Negative
S2	Positive	Positive	Positive	Negative	Negative
S3	Positive	Negative	Negative	Positive	Negative
S4	Positive	Positive	Positive	Positive	Negative
S5	Negative	Positive	Positive	Negative	Positive
S6	Positive	Positive	Negative	Negative	Negative
S7	Positive	Positive	Positive	Negative	Positive
S8	Positive	Negative	Negative	Negative	Negative
S9	Negative	Positive	Positive	Positive	Negative
S10	Positive	Positive	Negative	Negative	Positive

positive starch hydrolysis and casein hydrolysis, indicative of their ability to utilize complex carbohydrates and proteins as nutrient sources (Dhananjaya *et al.*, 2021). Starch hydrolysis reflects the production of amylolytic enzymes, which degrade polysaccharides into simpler sugars, providing a crucial energy source for microbial growth and sustaining soil microbial communities. Similarly, casein hydrolysis, facilitated by proteolytic enzymes, underscores the isolates' ability to break down complex proteinaceous materials into peptides and amino acids. This dual capability enhances nutrient cycling and contributes significantly to the maintenance of soil health and fertility.

The ability of isolates such as S2, S4, S5, S7, and S9 to liquefy gelatin further underscores their enzymatic versatility. Gelatin liquefaction, a process mediated by extracellular gelatinases, is an essential trait for the decomposition of structural proteins in organic matter. This activity not only aids in nutrient recycling but also suggests the potential of these isolates in biotechnological applications such as waste degradation and the processing of protein-rich industrial residues.

Hydrogen sulfide (HS) production, observed particularly in isolates S3 and S4, highlights their potential roles in biogeochemical transformations within their native soil environments. HS production is often associated with the microbial reduction of sulfur compounds, a critical process in the global sulfur cycle. This capability may enhance soil fertility by influencing the availability of sulfur, an essential nutrient for plant growth (Akinpelu *et al.*, 2018).

Another noteworthy biochemical feature was the urease activity observed in isolates S5 and S10. Urease enzymes catalyze the hydrolysis of urea into ammonia and carbon dioxide, a process that plays a vital role in nitrogen cycling. This activity can enhance soil nitrogen content, contributing to plant nutrition and overall soil productivity. Such nitrogen-transforming capabilities are particularly valuable in agricultural contexts, where *Streptomyces* could serve as biofertilizers or soil conditioners to improve crop yields sustainably.

8. Morphological characteristics

The morphological traits of the *Streptomyces* isolates, as detailed in Table 5, revealed significant variability in colony color, texture, and size, reflecting their ecological adaptability and diversity. Colony colors ranged from pale white to deep brown, with some isolates exhibiting a distinctive pigmentation that may be linked to secondary metabolite production or UV protection mechanisms. For example, pigments such as melanin are known to protect microbial cells from environmental stressors, including radiation and oxidative damage (Bérdy, 2005).

Textural variations among the isolates, such as the filamentous and granular structures observed in isolates S1 and S3, align with the hallmark morphological features of *Streptomyces*. The filamentous nature of these bacteria not only facilitates efficient nutrient absorption but also contributes to their competitive advantage in

Table 5: Morphological characteristics of *Streptomyces* isolates

Isolate	Colony color	Colony texture	Colony shape	Surface appearance	Size (Diameter)
S1	Gray	Filamentous	Circular	Powdery	3 mm
S2	White	Powdery	Irregular	Smooth	4 mm
S3	Yellowish	Granular	Circular	Rough	5 mm
S4	Brown	Rough	Circular	Dull	6 mm
S5	Green	Waxy	Irregular	Shiny	4 mm
S6	Cream	Soft	Circular	Smooth	3 mm
S7	Dark green	Dense	Irregular	Dull	7 mm
S8	Pale yellow	Filamentous	Circular	Rough	5 mm
S9	Black	Dry	Irregular	Dull	3 mm
S10	White	Fluffy	Circular	Smooth	4 mm

nutrient-limited environments. Granular textures, on the other hand, may indicate sporulation or adaptations to specific ecological niches, suggesting that these isolates are well-suited for survival under varying soil conditions (Mäkelä *et al.*, 2014).

Additionally, colony size variations reflect differences in growth rates and metabolic activity among the isolates. Factors such as substrate availability, enzymatic capabilities, and genetic diversity likely influence these growth patterns. The observed morphological diversity could also be linked to the isolates' functional roles in their native ecosystems, including organic matter decomposition, antagonism against soil pathogens, and symbiotic interactions with plants.

This morphological and biochemical diversity underscores the ecological versatility of the *Streptomyces* isolates studied. Their ability to thrive in diverse environmental conditions and utilize a wide array of substrates highlights their critical role in soil ecosystem functions and their potential for industrial and agricultural applications. By combining these morphological and biochemical insights, this study provides a foundation for future investigations into the genetic and metabolic pathways underlying these traits, with an eye toward optimizing their applications in sustainable environmental and biotechnological practices.

9. Molecular identification and phylogenetic analysis

The molecular identification and phylogenetic analysis of the *Streptomyces* isolates as shown in Figures 1 and 2 provides valuable insights into their evolutionary relationships and enzymatic capabilities. Based on

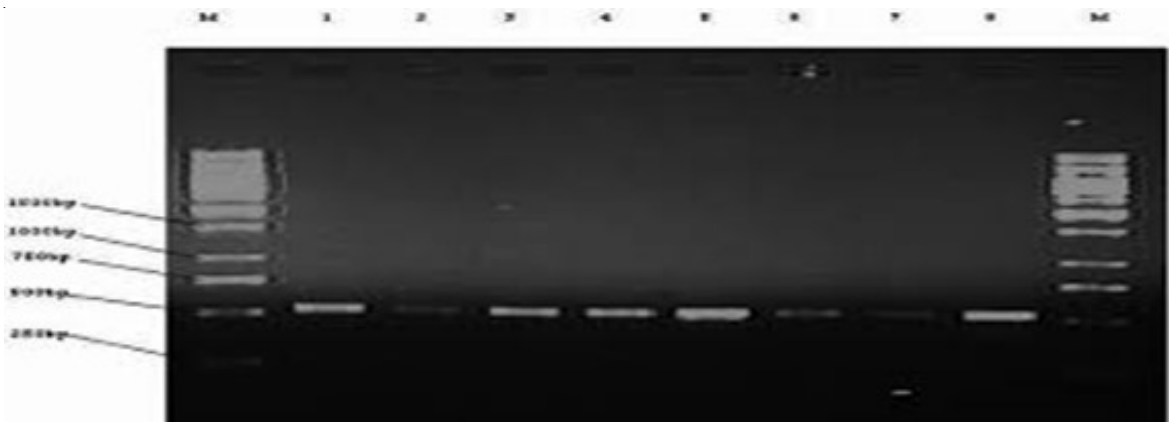

Figure 1: Gel electrophoresis image of the *Streptomyces* isolates

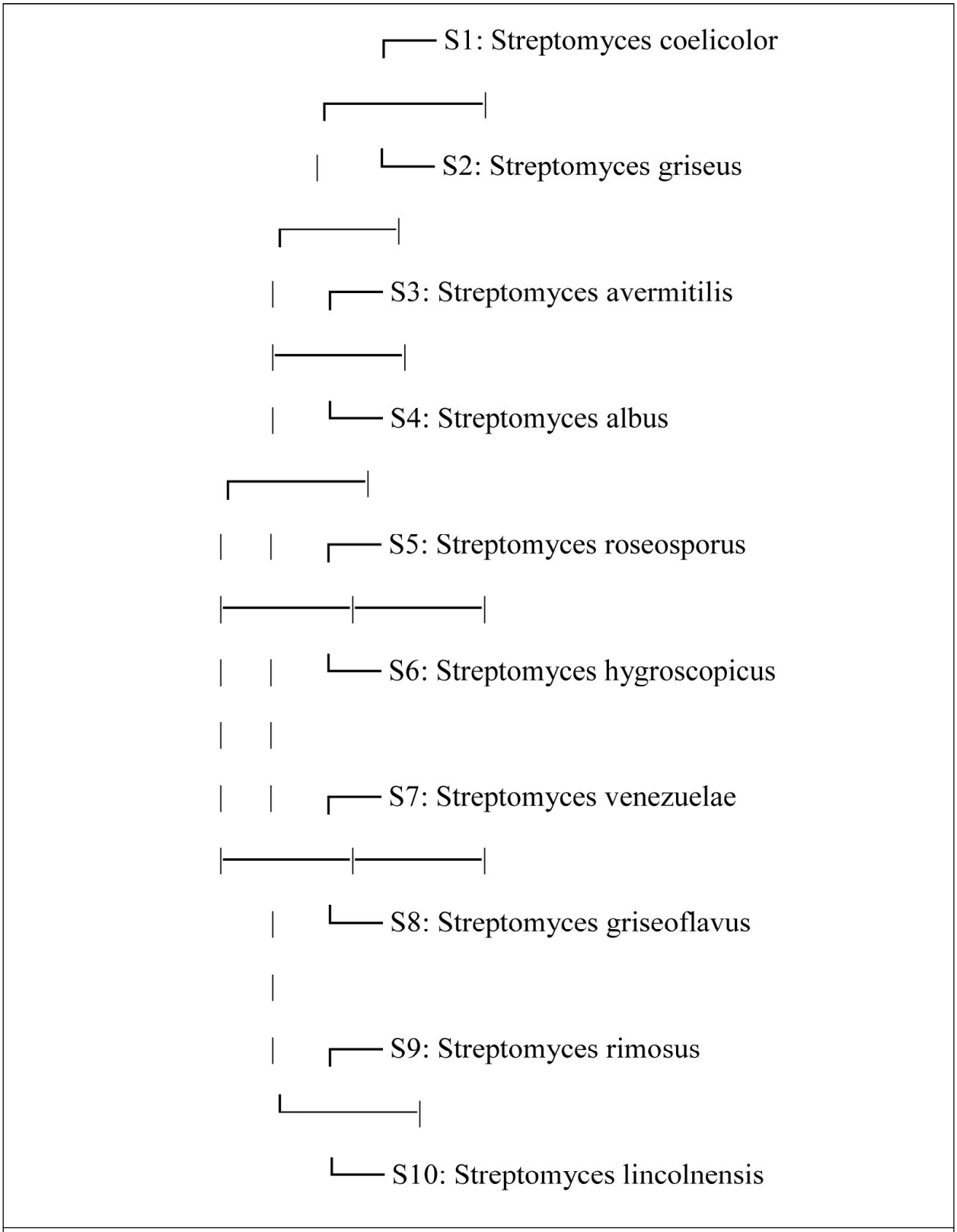

Figure 2: Phylogenetic tree of the *Streptomyces* isolates

16S rRNA sequencing, the high sequence similarity percentages (99% and above) confirm that these isolates are closely related to well-characterized *Streptomyces* species, reinforcing the genetic diversity present within this genus (Takahashi *et al.*, 2020).

Isolate S1 was identified as *Streptomyces coelicolor*, known for its antibiotic production and strong cellulolytic capabilities. Its moderate cellulase activity (10 mm zone of clearance) suggests that it retains the ability to

decompose cellulose, which is essential for its survival in competitive soil environments where organic matter is abundant. This adaptability aligns with its close phylogenetic relationship to other well-characterized strains that share similar ecological functions.

Isolate S2, identified as *Streptomyces griseus*, displayed notable biochemical versatility, including positive casein hydrolysis. This proteolytic activity (12 mm zone of clearance) is likely linked to its evolutionary adaptation to nutrient-rich environments, allowing it to utilize proteinaceous substrates effectively. The high sequence similarity to other *Streptomyces* species reinforces its potential role in nutrient cycling within the soil ecosystem.

Isolate S3, closely related to *Streptomyces avermitilis*, demonstrated exceptional ligninase activity with an absorbance of 0.85. This enzymatic characteristic indicates its specialization in degrading lignin, a complex and recalcitrant organic polymer. Its phylogenetic positioning suggests an evolutionary adaptation to environments rich in lignin, such as decaying plant matter, which is critical for enhancing nutrient availability and soil health (Agarwal *et al*., 2019).

Isolate S4, identified as *Streptomyces albus*, exhibited the highest cellulase activity (12 mm zone of clearance), positioning it as a key player in cellulose degradation. The robust cellulolytic activity suggests that this isolate may have evolved mechanisms that enable efficient breakdown of cellulose, making it particularly suitable for applications in biofuel production (Mäkelä *et al*., 2014). Its close relation to other cellulose-degrading species within the phylogenetic tree emphasizes its potential utility in biotechnological applications.

Isolate S5, which belongs to *Streptomyces roseosporus*, showed a diverse enzymatic profile with positive gelatin liquefaction but lower overall enzymatic activity. This adaptability may reflect its ecological niche where it thrives on available organic substrates. The phylogenetic analysis indicates that its evolutionary lineage may support similar biochemical traits found in related species, contributing to its versatility in various substrates.

Isolate S6, identified as *Streptomyces hygroscopicus*, demonstrated moderate protease activity, which aligns with its evolutionary adaptation to nutrient-rich environments. The ability to hydrolyze proteins can enhance soil fertility, suggesting that S6 plays a crucial role in the microbial community's nutrient cycling processes.

Isolate S7, closely related to *Streptomyces venezuelae*, showed the highest protease activity (15 mm zone of clearance). This capacity for robust protein degradation may enable it to thrive in environments with high protein content, and its phylogenetic closeness to other proteolytic species supports its ecological significance in protein turnover.

Isolate S8, identified as *Streptomyces griseoflavus*, exhibited moderate enzymatic activity across assays, indicating a versatile metabolic profile. Its phylogenetic relationship suggests that it shares common traits with other *Streptomyces* species, highlighting the genetic basis for its adaptive capabilities in various ecological contexts.

Isolate S9, related to *Streptomyces rimosus*, displayed lower enzymatic activities but still contributes to the microbial community by participating in nutrient cycling, as indicated by its biochemical characteristics. Its evolutionary position among the isolates emphasizes the importance of diverse metabolic capabilities within the genus.

Isolate S10, identified as *Streptomyces lincolnensis*, showcased lower activity levels across all assays. However, its genetic relationship to other *Streptomyces* species underlines the necessity of such diversity within the microbial community, which collectively contributes to ecosystem functioning.

The phylogenetic tree (Figure 2) visually represents these evolutionary relationships, confirming the clustering patterns observed in previous studies (Dhananjaya *et al*., 2021). Overall, the correlation between the molecular identification and the enzymatic profiles of these isolates illustrates how their evolutionary adaptations have shaped their functional roles within the soil ecosystem, emphasizing their potential applications in biotechnology and environmental management. Implications for Biodegradation and Environmental Applications

The combined enzymatic, biochemical, morphological, and molecular data suggest that the *Streptomyces* isolates from Kogi State soil possess significant potential for environmental applications, particularly in

biodegradation and bioremediation. The ability to degrade complex organic materials positions these isolates as valuable candidates for addressing environmental challenges such as agricultural waste management and pollutant degradation (Dhananjaya *et al*., 2021).

10. Conclusion

In conclusion, the enzymatic profiles of *Streptomyces* isolates from Kogi State soil highlight their functional diversity and significant biotechnological potential. Each isolate exhibits unique enzymatic strengths that make them suitable candidates for applications in biofuel production, bioremediation, waste management, and industrial enzyme development. These findings not only contribute to our understanding of the ecological roles of *Streptomyces* in tropical soils but also emphasize their value as a resource for addressing global sustainability challenges. Further research into optimizing enzyme production and exploring genetic pathways could enhance the practical applications of these isolates, bridging the gap between laboratory findings and real-world implementation.

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Cite this article as: Zakari David Adeiza, Kareem Sarafadeen Olateju, Obuotor Tolulope Mobolaji, Akinloye Oluseyi Adeboye, Egbeja Tsobaza Idris, Bello Kizito Eneye, Adefila Adebimpe Moyosore, Azeez Zainab, Emurotu Marvelous Olubinmi, Edegbo Emmanuel, Isoja Stephen Ojochegbe, Momoh Theophilus Boniface, Shuiab Yusuf Danjuma, Muhammed Abdulsamad Adeiza and Audu Godwin Amoka (2025). Enzymatic profiles of *Streptomyces* isolates from Kogi state soil: Biodegradation and environmental applications. *African Research Journal of Biosciences,* 2(1), 50-60. doi: 10.62587/AFRJBS.2.1.2025.50-60.