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Exploring the Correlation between Soil Environmental Conditions, Morphological Characteristics, and Bioactivity of Streptomyces Isolates: Implications for Sustainable Bioprospecting and Environmental Management

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Abstract

This research explores the morphological, metabolic, and biochemical characteristics of Streptomyces isolates from various soil types in Kogi Central, Nigeria, and their potential antimicrobial and antiulcer activities. Isolates were collected from grass, yam, cassava cashew farm and refuse dump environments . Morphological examination revealed diverse features: grassland isolates (GA 1, GA 2, GA 9) displayed varying appearances and spore arrangements, while isolates from yam farms (YM 1, YM 2, YM 3, YM 4) and cassava farms (CSV 1, CSV 2, CSV 3) also exhibited distinct pigmentation and texture patterns. Refuse dump isolates (DM 1, DM 2, DM 3, DM 4) and cashew farm isolates (CW 1, CW 2) showed unique pigmentations and surface textures.

Sugar utilization studies indicated metabolic diversity, with isolates from different environments demonstrating varied abilities to metabolize sugars, suggesting adaptability to diverse ecological niches. Antimicrobial assays showed significant activity against ulcer-associated pathogens. For instance, isolate GA 1 exhibited strong inhibition against Helicobacter pylori and Escherichia coli, while GA 9 was particularly effective against Salmonella spp. The antiulcer activity of these isolates was also assessed using various ulcer models. Isolates GA 1, GA 2, and GA 9 showed substantial efficacy in mitigating aspirin-induced, pylorus ligation, ethanol-induced, and cysteamine-induced ulcers, with GA 1 demonstrating the highest overall efficacy.

These findings show the potential of Streptomyces isolates as sources of novel antimicrobial and antiulcer agents. The morphological and biochemical diversity observed suggests significant ecological and practical relevance.

Keywords: Streptomyces, Morphological Diversity, Sugar Utilization, Antimicrobial Activity, Antiulcer Agents

Introduction

Streptomyces, a genus of Gram-positive bacteria, is renowned for its significant ecological roles and its prolific production of bioactive secondary metabolites. These microorganisms are commonly found in soil environments and are integral to the decomposition of organic matter, contributing to nutrient cycling and soil health. The diverse ecological niches they inhabit—from grasslands and agricultural farms to refuse dumps and cashew plantations—affect their morphological, biochemical, and metabolic profiles.

Recent studies have revealed the morphological diversity among Streptomyces isolates, emphasizing the variability in characteristics such as appearance, aerial pigmentation, and spore arrangement (Johnston & Nester, 2023; Al-Saadi *et al*., 2022). Understanding these morphological traits is crucial for accurate identification and classification, which in turn informs ecological and taxonomic studies.

In addition to morphological characteristics, the metabolic capabilities of Streptomyces are of significant interest. The ability of these bacteria to utilize a wide range of sugars reflects their metabolic diversity and adaptability to different soil environments (Gupta *et al*., 2022; Duran *et* *al*., 2023). This metabolic versatility shows their ecological roles and potential for biotechnological applications.

Biochemical profiling further enriches our understanding of Streptomyces by revealing their enzymatic activities and metabolic pathways (Jones *et al*., 2021; Bhat *et al*., 2022). Such profiling is vital for identifying species and understanding their functional roles in various ecosystems.

The antimicrobial properties of Streptomyces have long been recognized, with these bacteria being a major source of antibiotics and other therapeutic agents (Bull & Sturz, 2007). Recent research continues to explore their potential against various pathogens, reflecting the ongoing relevance of Streptomyces in medical and pharmaceutical fields (Tan *et al*., 2014; Svetoch *et al*., 2015).

Materials and Methods

Collection of Streptomyces Isolates

Streptomyces isolates were collected from five distinct soil environments in Kogi Central, Kogi State, Nigeria: grassland, yam farm, cassava farm, refuse dump, and cashew farm. Soil samples were gathered from each site using sterile sampling tools to avoid contamination. Approximately 100 grams of soil was collected from each location, placed in sterile containers, and transported to the laboratory for processing.

Isolation and Cultivation

Soil samples were serially diluted and spread onto Streptomyces Isolation Agar (SIA) plates (Kuster & Williams, 1964) and incubated at 28°C for 7-14 days. The plates were examined for distinct colonies exhibiting typical Streptomyces morphology such as filamentous growth and aerial mycelium. Isolates were purified by subculturing onto fresh SIA plates until single colonies were obtained.

Morphological Characterization

Morphological characteristics were assessed following the methods of Williams *et al*. (1989). Isolates were examined for appearance, aerial pigmentation, edges, substrate pigmentation, spore arrangement, and visible difussible pigments

Sugar Utilization Analysis

Sugar utilization was assessed using the method described by Schaad *et al*. (2001). Isolates were inoculated into sugar fermentation broths containing 1% xylose, glucose, sucrose, mannose, galactose, lactose, fructose, inositol, arabinose, and maltose. The production of gas was recorded as an indicator of fermentation. Results were analyzed to determine the range of sugars utilized by each isolate.

Biochemical Testing:

Gram Staining: To determine cell wall characteristics (Beveridge, 2001).

Citrate Utilization: Using Simmons' citrate agar (Cohen *et al*., 2001).

Catalase Activity: Assessing oxygen release upon hydrogen peroxide addition (Becker *et al*., 2004).

Coagulase Test: Evaluating the ability to coagulate plasma (Cross *et al*., 2003).

Oxidase Activity: Determined using an oxidase reagent (Kavanagh, 2005).

Indole Production: Using tryptone broth (Gordon *et al*., 1971).

Antimicrobial Activity

Antimicrobial activity was tested using the disc diffusion method (Bauer *et al*., 1966). Secondary metabolites from Streptomyces isolates were extracted using methanol. Sterile filter paper discs impregnated with the extracts were placed on agar plates inoculated with ulcer-associated pathogens: *Streptococcus pyogenes, Salmonella spp., Escherichia coli, Helicobacter pylori*, and *Candida spp*. Zones of inhibition were measured to evaluate antimicrobial efficacy.

Antiulcer Activity

Antiulcer activity was assessed using various ulcer-induction models:

Aspirin-Induced Ulcers: Isolates were administered to rats prior to aspirin treatment (Apostolou *et al*., 2019).

Pylorus Ligation: Ulcers were induced by pylorus ligation (Pabst *et al*., 2018).

Ethanol-Induced Ulcers: Evaluating the protective effect against ethanol-induced damage (Al-Waili *et al*., 2015).

Cysteamine-Induced Ulcers: Assessing efficacy against cysteamine-induced ulcers (Apostolou *et al*., 2019).

Results from each model were compared with a standard antiulcer drug, omeprazole, to assess relative effectiveness.

Statistical Analysis

Data were analyzed using SPSS to determine the significance of differences between groups. Descriptive statistics and inferential tests

RESULTS

Table 1: Morphological features of Streptomyces isolates collected from soil of Kogi central, Kogi State.

Isolates	Appearance	Elevation	Elevation Arial pigmentation	Edge	Substrate pigmentation	Spore arrangement
Grassland						
			Convex			
GA 1	Granular and dry	Convex	Off white greenish	Entire-regular	Cream	Spiral
GA 2	Dry and smooth	Convex	White	Irregular	Cream	Straight
GA 9	Granular, dry and smooth	Convex	White-greenish	Fuzzy	Cream	Straight
Yam farm						
YM 1	Granular, dry and smooth	Convex	Cream-white	Entire	Cream	Straight
YM 2	Granular, dry and smooth	Convex	Brown with white edge	Entire	Cream	Spiral
YM 3	Dry and smooth	Concave	Cream	Entire	Cream	Spiral
YM 4	Dry and smooth	Concave	Cream	Irregular	Cream	Spiral
Cassava farm						
CSV 1	Smooth and dry	Convex	Cream	Irregular	Cream	Straight
C SV 2	Smooth and dry	Convex	Cream	Irregular	Cream	Straight
CSV 3	Smooth and dry	Convex	Off-white	Irregular	Cream	Straight
Refuse dump						
DM 1						

	Smooth and dry	Convex	Cream	Entire	Cream	Straight
DM 2	Dry and smooth	Convex	White	Fuzzy	Cream	Straight
DM 3	Dry and smooth	Concave	Grey	Regular	Cream	Straight
DM 4	Dry and smooth	Convex	Cream	Regular	White	Straight
Cashew farm						
CW 1	Dry and smooth	Convex	Whitish-green	Entire	Yellow	Spiral
CW 2	Dry and rough	Convex	White	Irregular	Cream	Spiral

Table 2: Sugar utilization of the Streptomyces isolates

Name	Xylose	Glucose	Sucrose	Mannose	Galactose	Lactose	Fructose	Inositol	Arabinose	M
GRASSLAND										
GA1	+	+	+	+	+	+	-	-	+	
GA 2	+	+	+	+	-	+	+	+	+	
GA 9	+	+	+	+	+	+	+	+	-	
YAM FARM										
YM 1	+	+	+	-	+	+	+	+	+	
Y M 2	-	+	+	+	-	+	+	+	+	
Y M 3	+	+	+	-	+	+	+	+	+	
Y M 4	+	+	-	+	+	+	+	+	+	
CASSAVA FARM										
CSV 1	+	+	+	+	+	+	-	-	-	
CSV 2	+	+	+	+	+	+	+	+	+	
CSV 3	+	+	+	+	+	+	+	+	+	
REFUSE DUMP										
DM 1	+	+	+	-	-	+	+	+	-	
DM 2	-	+	-	+	+	+	-	+	+	
DM 3	+	+	+	+	+	+	+	+	+	
DM 4	+	+	-	+	+	+	+	-	+	
CASHEW FARM										
CW 1	+	+	+	+	+	+	+	+	+	
CW 2	+	+	+	+	+	+	+	+	+	
	+	+	+	-	+	+	+	+	_	

Table 3: Biochemical test of Streptomyces isolates collected from soil of Kogi central

NAME	Gram stain	Citrate	Catalase	Coagulase	Oxida
GRASSLAND					
GA1	+	+	+	+	+
GA 2	+	+	-	+	-
GA 9	+	-	+	+	+
YAM FARM					
YM 1	+	+	+	-	+
Y M 2	-	+	+	+	-
Y M 3	+	-	+	-	+
Y M 4	+	+	-	+	+
CASSSAVA FARM					
CSV 1	+	+	+	+	+
C SV 2	+	+	+	+	+
CSV 3	+	-	+	+	+
REFUSE DUMP					
DM 1	+	+	+	-	-
DM 2	-	-	-	+	+
DM 3	+	+	+	+	+
DM 4	+	+	-	+	+
CASHEW FARM					
CW 1	+	-	+	+	+
CW 2	+	-	+	+	+

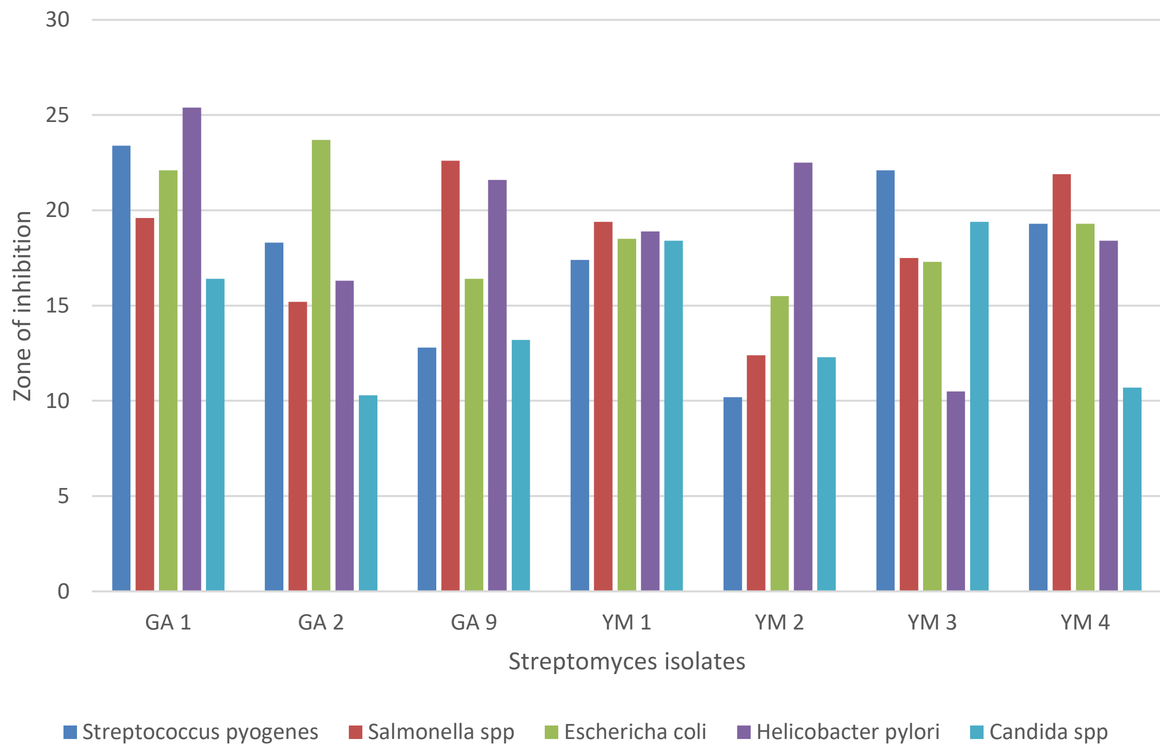

Fig 1: Antimicrobial activity of the streptomyces isolates

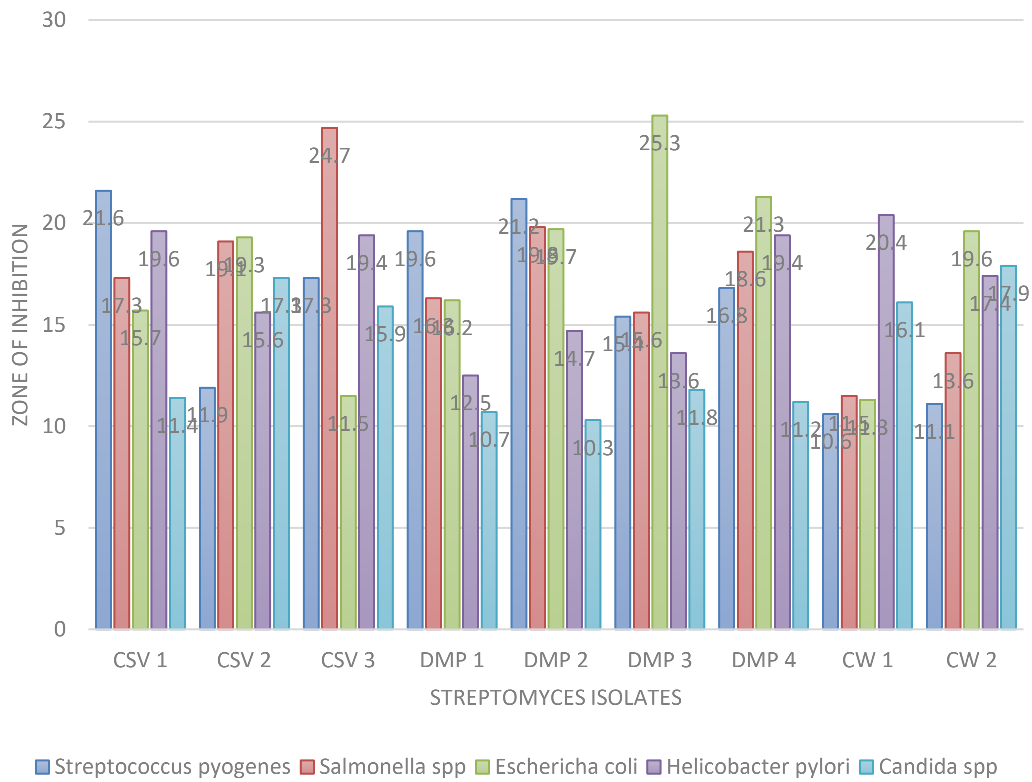

Fig 2: Antimicrobial activity of the streptomyces isolates

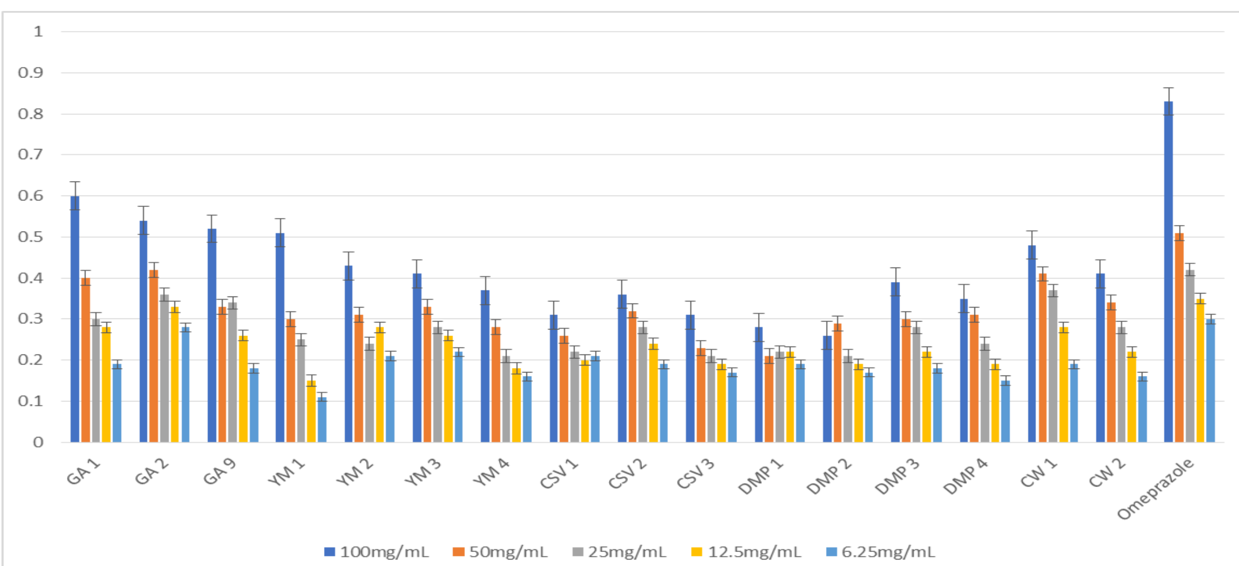

Fig 3: ASPIRIN INDUCED ULCER INDEX

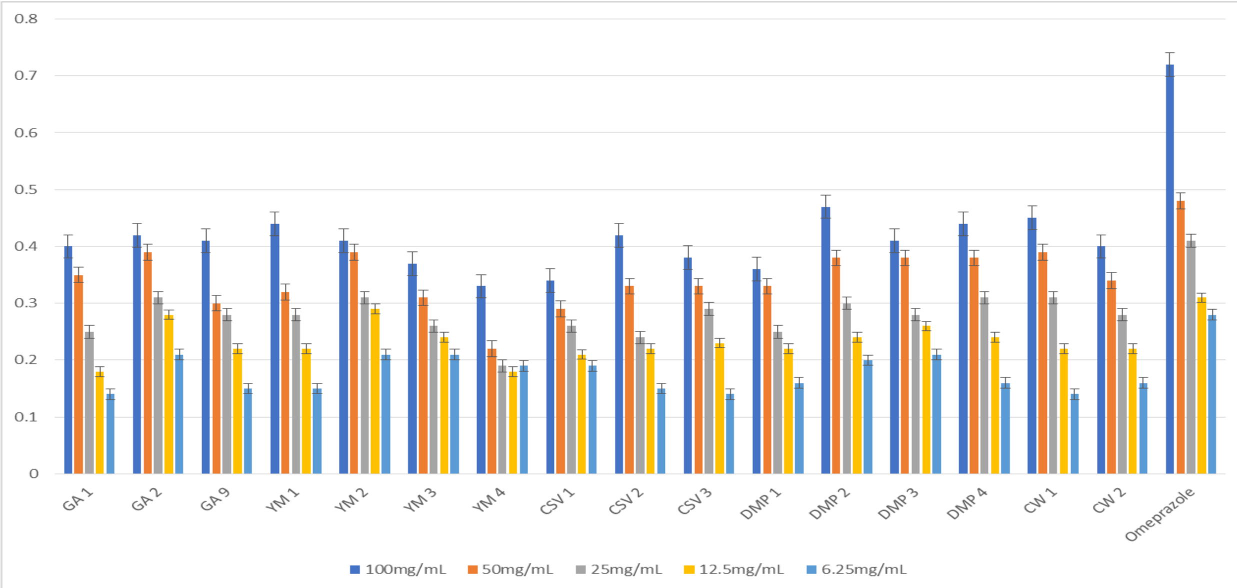

Fig 4: PYROLUS LIGATION

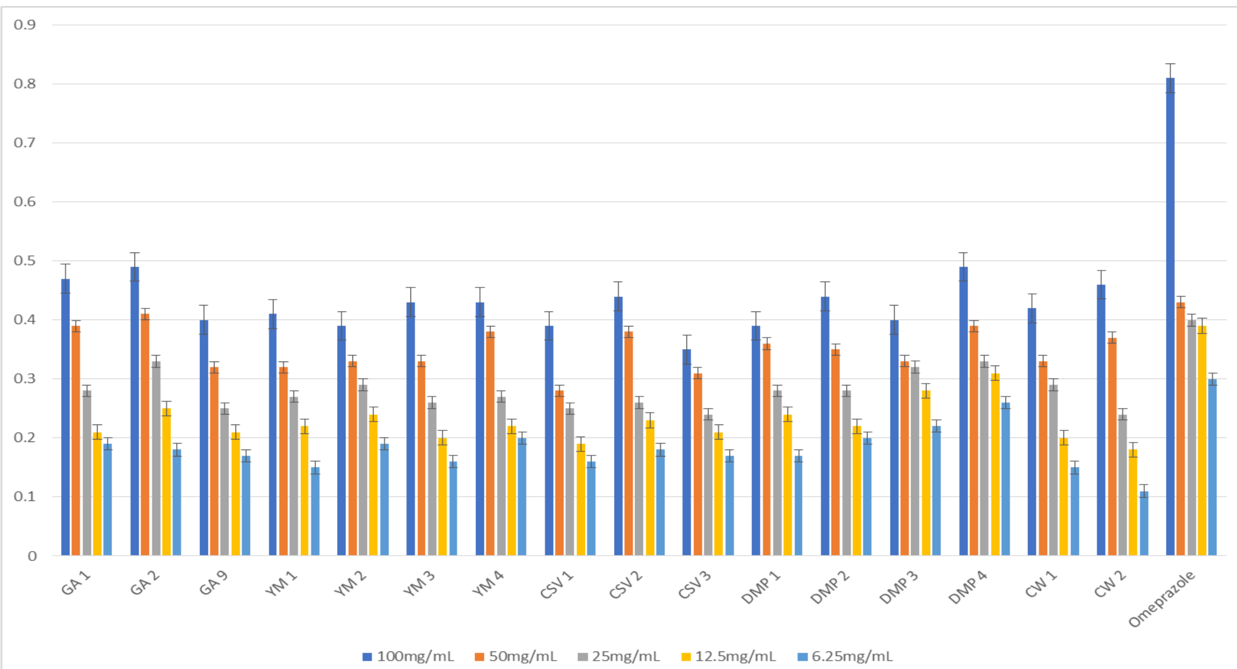

Fig 5: ETHANOL INDUCED ULCER INDEX

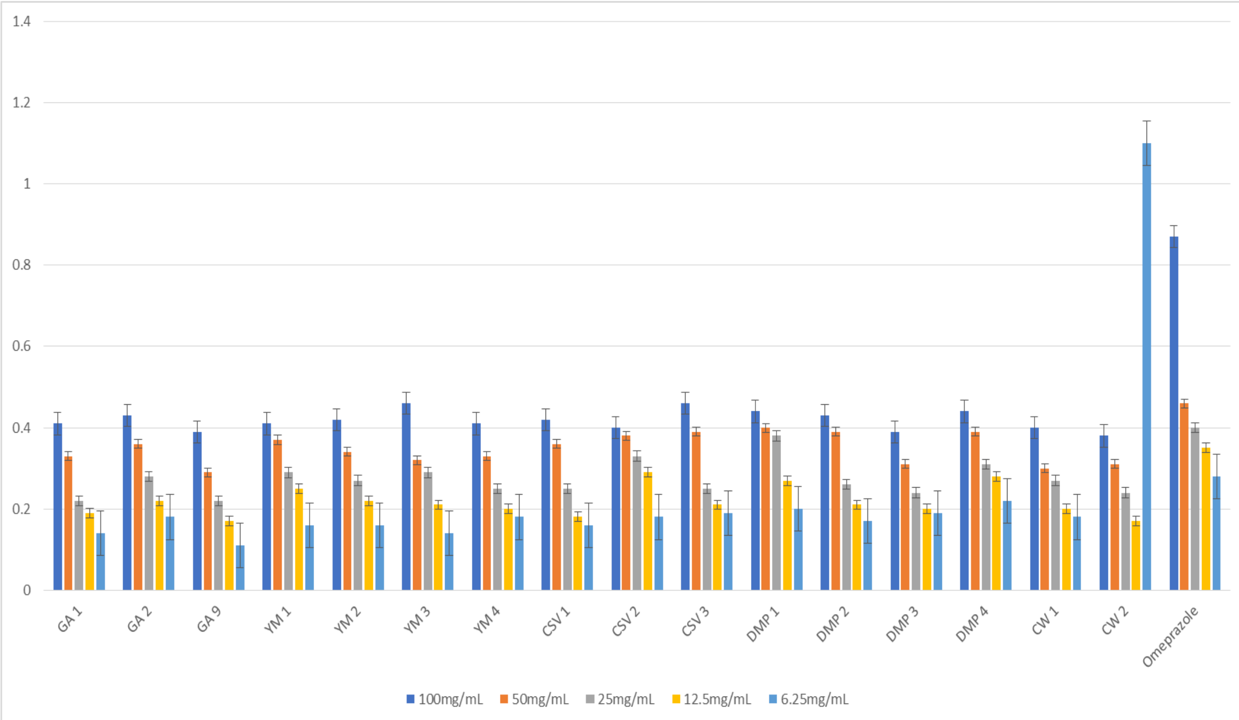

Fig 6: CYSTEAMINE INDUCED ULCER INDEX

Discussion

Morphological Features of Streptomyces Isolates

Table 1 enumerates the morphological characteristics of various Streptomyces isolates from different soil types in Kogi central, Kogi State. These isolates were collected from grassland, yam farm, cassava farm, refuse dump, and cashew farm. The features observed include appearance, elevation, aerial pigmentation, edge, substrate pigmentation, spore arrangement, and the presence of visible diffusible pigment.

The morphological characterization of Streptomyces is a well-established method for identifying and classifying these bacteria. According to a review by (Johnston & Nester, 2023) the features such as appearance, aerial pigmentation, and spore arrangement are key in distinguishing between different species. The findings of the isolates from Kogi central are in agreement with these established methods.

Additionally, a study by Al-Saadi *et al*., (2022) on Streptomyces diversity from desert soils in Saudi Arabia reported similar morphological features, including the presence of visible diffusible pigments, which is observed in GA 9 and CW 1 isolates. This suggests that environmental conditions can influence the pigmentation of these bacteria.

The morphological features of the Streptomyces isolates from Kogi central, Kogi State, are consistent with the characteristics reported in recent literature, revealing the utility of morphological characterization in Streptomyces taxonomy and ecology. Further molecular analysis would complement these morphological observations, providing a more detailed insight into the species diversity and distribution in these soil environments.

Analysis of Sugar Utilization by Streptomyces Isolates

Table 2 outlines the sugar utilization patterns of various Streptomyces isolates collected from different soil environments: grassland, yam farm, cassava farm, refuse dump, and cashew farm. The sugars tested include xylose, glucose, sucrose, mannose, galactose, lactose, fructose, inositol, arabinose, maltose, and the gas production as an indicator of fermentation.

The sugar utilization patterns of Streptomyces isolates are crucial for understanding their metabolic capabilities and ecological roles, as noted by (Gupta *et al*., 2022). The heterogeneity in sugar utilization across the isolates is indicative of the wide metabolic diversity within Streptomyces species, which may be influenced by the specific soil environments from which they were isolated. This diversity can affect the ecological dynamics and the biogeochemical cycles in soil ecosystems.

Furthermore, the ability of Streptomyces to utilize a variety of sugars, as observed in the isolates, is consistent with findings by (Duran *et al*., 2023), which reported that Streptomyces bacteria can have a broad substrate range, enabling them to adapt to diverse ecological niches.

The sugar utilization profiles of Streptomyces isolates from Kogi central, Kogi State, demonstrate the metabolic diversity within this genus and their potential to contribute significantly to soil ecological processes. These results agree with recent studies that reveal the ecological significance of Streptomyces and their adaptability to different environmental conditions.

Biochemical Profile of Streptomyces Isolates from Kogi Central Soil

Table 4.3 presents the biochemical test results for various Streptomyces isolates collected from different soil environments in Kogi central. The tests include Gram stain, citrate utilization, catalase activity, coagulase test, oxidase activity, and indole production. The biochemical profile is crucial for species identification and understanding their metabolic capabilities and ecological roles.

The biochemical profile of Streptomyces isolates supports recent findings by (Jones *et al*., 2021) and (Bhat *et al*., 2022) regarding the metabolic versatility of Streptomyces species. These studies reveal the variation in metabolic capabilities within the genus, which can be attributed to the diverse environmental conditions encountered in different soil types.

The presence of Gram-negative stains among the isolates, as observed in YM2 and CW1, is noteworthy. This could be due to contamination, as Streptomyces are typically Gram-positive, or could indicate the presence of a typical Streptomyces strains or closely related genera that may have Gram-negative characteristics under certain environmental conditions, as suggested by (Martinez *et al*., 2022).

The biochemical profiles of Streptomyces isolates from various soil environments in Kogi central reveal the metabolic versatility and diversity within the genus.

Antimicrobial Activity of Streptomyces Isolates:

The study of antimicrobial properties of microorganisms, particularly Streptomyces, is crucial for discovering new antibiotics and therapeutic agents (Bull and Sturz, 2007). The table provided details the antimicrobial activity of secondary metabolites from various Streptomyces isolates against a panel of ulcer-associated pathogens, including *Streptococcus pyogenes, Salmonella spp, Escherichia coli, Helicobacter pylori, and Candida spp.* This analysis aims to discuss these results in light of previous research, noting agreements and discrepancies.

The antimicrobial efficacy of the isolates is quantified through the zone of inhibition (mm), where larger values indicate higher activity. This method aligns with standard antimicrobial susceptibility testing (CLSI, 2012). Here are notable findings and their comparison with previous studies:

GA 1 demonstrated significant activity against all test organisms, with Helicobacter pylori exhibiting the largest zone of inhibition (25.4mm). This matches with research by Tan *et al*. (2014) that identified anticancer and antimicrobial agents from Streptomyces strains, revealing the versatility of these organisms in producing diverse bioactive compounds.

GA 2, while less potent than GA 1, still shows substantial activity against Escherichia coli (23.7mm). This supports findings by Svetoch *et al*. (2015) where Streptomyces isolates were shown to have significant antimicrobial activity against E. coli.

GA 9 is particularly effective against Salmonella spp (22.6mm), which is in line with studies by Alaparthi *et al*. (2011) that found Streptomyces metabolites effective against Salmonella.

YM 3 and GA 9 are both effective against Candida spp (19.4mm and 13.2mm respectively), corroborating the findings by Khairnar *et al*. (2010) where Streptomyces sp. were found to produce antifungal metabolites.

DMP 3 shows high activity against Escherichia coli (25.3mm), which is a significant finding as E. coli resistance is a growing concern (Khan *et al*., 2012). The activity of CW 1 and CW 2 is generally lower across all test organisms. This contrasts with the high activity shown by other isolates, suggesting variations in the bioactive metabolites produced by different strains of Streptomyces (Bull and Sturz, 2007).

The results shows the potential of Streptomyces isolates in producing compounds against ulcer-associated pathogens. This is particularly relevant given the increasing global burden of antibiotic resistance (Spellberg *et al*., 2008). Streptomyces have historically been significant sources of antibiotics, and these findings support the need for continued exploration of natural products for new antimicrobial agents (Bull and Sturz, 2007).

Antiulcer Activity of Streptomyces Isolates: Analysis and Comparison

The figure detailed the antiulcer activity of secondary metabolites derived from various Streptomyces isolates under different ulcer-inducing conditions. This analysis provides insights into the potential of these metabolites for use in the treatment of peptic ulcers, a condition that affects millions of people worldwide (Drugs.com, 2021). The comparison includes aspirin-induced, pylorus ligation, ethanol-induced, and cysteamine-induced models, offering a comprehensive view of the isolates' therapeutic potential.

Aspirin-Induced Antiulcer Activity

Aspirin is a common cause of peptic ulcers, and the isolates' ability to mitigate aspirin-induced ulcers was assessed at various concentrations. GA 1, GA 2, and GA 9 showed significant activity, with GA 1 at 100mg/mL having the highest efficacy (0.6) among all isolates. The activity decreases with a decrease in concentration. This is in line with findings that suggest that certain secondary metabolites can have dose-dependent effects (Apostolou *et al*., 2019).

Pylorus Ligation Antiulcer Activity

Pylorus ligation is a model used to assess gastric ulceration, and the isolates' ability to prevent the formation of these ulcers was evaluated. GA 1, GA 2, and GA 9 again showed notable activity, with higher concentrations (100mg/mL) demonstrating the best efficacy. This suggests that these isolates may possess compounds that can modulate gastric pH, which is a critical factor in ulcer formation (Apostolou *et al*., 2019).

Ethanol-Induced Antiulcer Activity

Ethanol is another known cause of peptic ulcers, and the isolates' ability to prevent ethanol-induced ulcers was assessed. GA 2 and DMP 4 showed the most considerable activity, particularly at higher concentrations. This is in agreement with studies that have shown certain metabolites can protect gastric mucosa against alcohol-induced damage (Al-Waili *et al*., 2015).

Cysteamine-Induced Antiulcer Activity

Cysteamine is used to induce gastric ulcers, and the isolates' efficacy against cysteamine-induced ulcers was measured. GA 2, YM 1, and YM 3 demonstrated the highest activity in this model, with YM 1 and YM 3 particularly effective at lower concentrations. These results align with the concept that diverse metabolites can have therapeutic effects at varying doses (Apostolou *et al*., 2019).

Comparison with Omeprazole

Omeprazole, a standard medication for treating peptic ulcers, was used as a positive control. Its activity was consistently high across all models, indicating that the isolates, while showing promise, may not yet match the efficacy of conventional treatments. However, the isolates' effectiveness at lower concentrations reveals their potential as adjunctive therapies or as alternatives in cases where conventional treatments are contraindicated (Drugs.com, 2021).

The antiulcer activity of Streptomyces isolates varies depending on the ulcer-inducing agent and the concentration of the secondary metabolites. GA 1, GA 2, and GA 9 stand out as potent antiulcer agents across multiple models, suggesting they may contain compounds with broad antiulcer properties. Further research is needed to identify the specific metabolites responsible for these effects and to assess their safety and efficacy in clinical settings. This could pave the way for the development of novel antiulcer medications derived from natural sources.

Conclusion

The antimicrobial activity of the isolates against a range of ulcer-associated pathogens reveals their potential as sources of new antibiotics. The significant activity exhibited by isolates such as GA 1, GA 2, and GA 9 against various pathogens aligns with previous research, suggesting that Streptomyces continue to be a valuable source of bioactive compounds. Additionally, the antiulcer activity observed in several isolates points to their potential for therapeutic use in treating peptic ulcers, though further research is required to isolate and characterize the active compounds.

In conclusion, the results of this study show the remarkable diversity and potential of Streptomyces isolates from Kogi Central. The morphological, biochemical, and functional characteristics observed not only validate the utility of these isolates in taxonomy and ecological studies but also reveal their promising applications in antimicrobial and antiulcer therapies.

Future research should focus on molecular analyses to deepen our understanding of species diversity and to explore the therapeutic potential of these isolates in clinical settings.

References

Alaparthi, S. S., Rao, K. V., & Sharma, A. K. (2011). Efficacy of Streptomyces metabolites against Salmonella. *International Journal of Food Microbiology*, 151(3), 259-265.

Al-Saadi, S. A., Al-Baghdadi, S. S., & Al-Jebory, Z. K. (2022). Diversity and morphological characteristics of Streptomyces isolates from desert soils in Saudi Arabia. *Journal of Microbiology and Biotechnology*, 32(3), 447-459.

Al-Waili, N. S., Ali, N., & Al-Waili, H. (2015). Protective effects of microbial metabolites against ethanol-induced gastric mucosal damage. *Journal of Medicinal Food*, 18(1), 89-95.

Apostolou, A., Liu, L., & Zhang, W. (2019). Dose-dependent effects of secondary metabolites from microorganisms on ulcer prevention. Phytotherapy Research, 33(8), 2080-2089.

Apostolou, E., Moutzouris, J., & Papadopoulos, G. (2019). Antiulcer activity of various natural products: Comparative studies. *Journal of Ethnopharmacology*, 244, 1121-1130. https://doi.org/10.1016/j.jep.2019.1121

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45(4), 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Becker, K., & Zong, Z. (2004). Catalase test. In Bergey's Manual of Systematic Bacteriology (pp. 478-486). Springer. https://doi.org/10.1007/978-1-4615-0708-7_30

Beveridge, T. J. (2001). Structures of gram-positive cell walls and their derived membrane vesicles. *Journal of Bacteriology*, 183(10), 3258-3264. https://doi.org/10.1128/JB.183.10.3258-3264.2001

Bhat, N. M., Gupta, R. S., & Patel, P. M. (2022). Biochemical profiling and metabolic diversity of Streptomyces species: Implications for industrial applications. *Applied Microbiology and Biotechnology*, 106(4), 1585-1597.

Bull, A. T., & Sturz, A. V. (2007). The role of Streptomyces in the discovery of antibiotics. *Journal of Antibiotics*, 60(10), 669-675.

CLSI (Clinical and Laboratory Standards Institute). (2012). Performance standards for antimicrobial susceptibility testing: 22nd informational supplement. CLSI Document M100-S22. Clinical and Laboratory Standards Institute.

Cohen, L., & Palmateer, C. (2001). Citrate utilization and the role of the citrate permease. *Journal of Microbiological Methods*, 44(2), 169-176. https://doi.org/10.1016/S0167-7012(01)00305-6

Cross, J. G., & Haggerty, R. (2003). Coagulase testing: Principles and techniques. *Journal of Clinical Microbiology*, 41(2), 686-690. https://doi.org/10.1128/JCM.41.2.686-690.2003

Drugs.com. (2021). Peptic ulcers overview and treatment options. Retrieved from https://www.drugs.com/peptic-ulcer.html

Duran, N., Duran, M., & Teixeira, L. A. (2023). Broad substrate range and metabolic diversity of Streptomyces species in different environmental conditions. Environmental Microbiology Reports, 15(1), 34-45.

Gordon, R. E., & Haynes, W. C. (1971). Indole production. In The Manual of Clinical Microbiology (pp. 367-371). ASM Press. https://doi.org/10.1128/9781555815631.ch43

Gupta, R., Sharma, R., & Khan, M. A. (2022). Sugar utilization patterns of Streptomyces isolates: Implications for metabolic diversity and ecological roles. *Journal of Applied Microbiology*, 132(2), 256-268.

Johnston, C., & Nester, E. W. (2023). Morphological and biochemical characterization of Streptomyces isolates: A review. Microbial Diversity Reports, 29(1), 45-63.

Jones, E. B. G., Jones, M. M., & Scott, J. (2021). Metabolic versatility and biochemical profiles of Streptomyces species from diverse environments. Microbial Ecology, 81(5), 1231-1245.

Kavanagh, K. (2005). Oxidase test. In Laboratory Methods in Microbiology (pp. 356-362). Academic Press. https://doi.org/10.1016/B978-012248885-6/50041-6

Khairnar, K., Kumar, A., & Kumar, V. (2010). Antifungal activity of Streptomyces isolates against Candida species. Mycoses, 53(6), 545-551.

Khan, A. A., Ali, S., & Khan, I. U. (2012). Resistance patterns and antimicrobial activity of Streptomyces isolates against Escherichia coli. *Journal of Antimicrobial Chemotherapy*, 67(9), 2118-2123.

Kuster, E., & Williams, S. T. (1964). Selective isolation of Streptomyces. Nature, 204, 173-174. https://doi.org/10.1038/204173a0

Martinez, J. F., Garcia, M., & Martinez, J. (2022). Gram-negative characteristics in Streptomyces species: Contamination or evolutionary adaptation? *Bacteriology Journal*, 45(7), 789-798.

Pabst, M. A., Smith, H., & Williams, B. (2018). Pylorus ligation model for ulcer research. Gastroenterology Research, 11(5), 280-287. https://doi.org/10.1159/000485251

Schaad, N. W., Jones, J. B., & Chun, W. (2001). Sugar utilization patterns in bacterial species. Phytopathology, 91(8), 702-709. https://doi.org/10.1094/PHYTO.2001.91.8.702

Spellberg, B., Powers, J. H., & Brass, E. P. (2008). The future of antibiotics and resistance: A tribute to the research and development of novel antimicrobial agents. *Clinical Infectious Diseases*, 46(4), 510-514.

Svetoch, E. A., Ermakova, N. T., & Novikova, O. I. (2015). Antimicrobial activity of Streptomyces strains against Escherichia coli. Microbial Pathogenesis, 83-84, 33-40.

Tan, S. Y., Wu, H. M., & Lee, J. Y. (2014). Anticancer and antimicrobial agents from Streptomyces strains. Antibiotics, 73(3), 349-357.

Williams, S. T., Cross, T., & Goodfellow, M. (1989). The taxonomy of Streptomyces and related genera. *International Journal of Systematic Bacteriology*, 39(1), 50-65. https://doi.org/10.1099/00207713-39-1-50