JFBSPR
Open Access

JFBSPR

Journal of Frontiers in Biological Sciences and Pharmacology Research

Available at: https://journals.pafacademicpublishers.com/jfbspr

Frontiers

Antibacterial Potential of Rhizosphere-Derived Actinomycetes from Millet (Pennisetum glaucum) in Jalingo, Nigeria

Jesse Innocent Apameio[1], Benson Terer Targema[*1], Abdulmumini Buba[1], Hassan Andetsiki[1], Mafe Alice Njolke[1], Oloninefa Stephen Dare[2], Attah Friday[3], Theophilus Istiphanus[1]

[1]Department of Biological Sciences, Taraba State University, Jalingo, Taraba State, Nigeria

[2]Department of Biological Sciences, Kogi State University, Kabba, Kogi State, Nigeria

[3]Department of Microbiology, Confluence University of Science and Technology, Osara, Kogi State, Nigeria

Keywords

Actinomycetes,
Isolation,
Millet,
Rhizosphere,
Antibiotic.

Abstract

This study assessed antibiotics produced from actinomycetes isolated from the rhizosphere of millet plants. Soil samples were collected from the rhizosphere of millet plants grown in agricultural fields within Jalingo metropolis. Each sample was collected from the top 10-15 cm of soil surrounding the millet roots to ensure the inclusion of the rhizosphere microbiome. Soil samples were air-dried and sieved through a 2 mm mesh. Actinomycetes were isolated using Actinomycete Isolation Agar. Isolates showing significant antibacterial activity in preliminary screening were subjected to secondary screening. The antibiotic activity was confirmed using well-diffusion assays. Antibiotics were extracted from the culture broth sediment after centrifugation. Streptomyces spp. displayed higher antibiotic activity, particularly on Day 21, with inhibition zones of 35 mm, 50 mm and 20 mm for E. coli, Salmonella sp. and Staphylococcus aureus, respectively. Nocardia spp. demonstrated moderate activity only on Days 14 and 21, and similarly Micromonospora sp. This study demonstrates the antimicrobial potential of Streptomyces spp. and Nocardia spp. against clinically significant pathogens, including Salmonella spp., Staphylococcus aureus, and Escherichia coli. In comparison, both Actinomycetes exhibited measurable inhibition; Streptomyces spp. generally showed higher efficacy against Salmonella spp. and E. coli, whereas Nocardia spp. was more effective against S. aureus.

1. Introduction

Actinomycetes, particularly those belonging to the genus Streptomyces, are the source of the majority of now-recognised antimicrobials. All significant medication families utilised in clinics today, such as β-lactams, tetracyclines, macrolides, aminoglycosides, and glycopeptides, are generated by these bacteria. (Goodfellow and Fiedler, 2010; Wang et al., 2020). However, in recent years, the effectiveness of these impressive antibiotics has been compromised by the rise of resistance among life-threatening pathogenic bacteria

*Corresponding Author: Benson Terer Targema
Email Address: bensontargema@gmail.com

(Ventola, 2015). Additionally, the golden era of antibiotic discovery has long passed, and there have been significant concerns about the antibiotic pipeline potentially running dry (Molloy, 2021).

Next-generation sequencing techniques combined with genome mining approaches have revolutionised the field of antibiotic research and offer hope for rejuvenating the pipeline in the near future (Baltz, 2017). In 2002, the first Streptomyces genome sequence was published, specifically that of the model actinomycete Streptomyces coelicolor (Bentley et al., 2002). Mining this sequence revealed that S. coelicolor harbours 22 secondary metabolite gene clusters, but it produces only four of the encoded metabolites under standard laboratory conditions (Bentley et al., 2002). As of now, more than 625 genome sequences are available for the genus Streptomyces alone (Cimermancic et al., 2014).

Antibiotics produced by actinomycetes are crucial in combating bacterial infections and have significantly impacted the field of medicine. Actinomycetes, a group of Gram-positive bacteria, are known for their diverse metabolic capabilities and prolific production of bioactive compounds (Goodfellow and Fiedler, 2010). Among these, antibiotics are particularly valuable due to their ability to inhibit the growth of pathogenic microorganisms, which makes them essential in both clinical and agricultural settings.
The rhizosphere of plants, including millet, serves as a rich source of actinomycetes. The complex interactions between plants and soil microorganisms often result in the presence of bacteria with novel properties (Berg et al., 2017). Millet is a staple crop with a significant rhizosphere microbiome, which can be a potential reservoir for discovering new antibiotic-producing actinomycetes (López-Fernández et al., 2019).

2. Materials and Methods

2.1 Sample Collection

2.1.1 Soil sampling

Soil samples were collected from the rhizosphere of millet plants grown in agricultural fields. Random sampling was performed to ensure representative samples. Each sample was collected from the top 10 to 15 cm of soil surrounding the millet roots to ensure the inclusion of the rhizosphere microbiome. The collected soil samples were placed in sterile containers, labelled, and transported to the laboratory under cold conditions to maintain microbial viability.

2.1.2 Sample Preparation

Soil samples were air-dried, sieved through a 2 mm mesh to remove debris, and mixed thoroughly to create a homogeneous sample for microbial analysis (Fiedler et al., 2018).

2.2 Isolation of Actinomycetes

2.2.1 Media preparation

Actinomycetes were isolated using the Actinomycete Isolation Agar (AIA). The medium was prepared according to the manufacturer's instructions and sterilised by autoclaving at 121°C for 15 minutes. Nystatin were added to inhibit the growth of fungi (Fiedler et al., 2018).

2.2.2 Isolation procedure

Soil samples were serially diluted in sterile phosphate-buffered saline (PBS), and aliquots of the diluted soil suspensions were spread onto the surface of the media. Plates were incubated at 28°C for 3-7 days. Colonies exhibiting distinctive morphology of actinomycetes being filamentous, were selected for further analysis.

2.3 Preliminary Screening

Pure cultures of actinomycetes were streaked in straight lines across the agar plate, near one edge. The plates were incubated at 30°C for 5 days. This allows the actinomycetes to grow and potentially produce antibiotics. After the initial incubation, test microorganisms (Salmonella species, Staphylococcus aureus and Escherichia coli) were streaked perpendicularly to the actinomycete streaks and the plates were incubated again at 37°C. After incubation, the plates were examined for clear zones of inhibition where the growth of the test microorganism were suppressed near the actinomycete streak.

2.4 Antibacterial susceptibility test of actinomycetes producing antibiotics

Isolates showing significant antibacterial activity in preliminary screening were subjected to secondary screening. Each isolate was cultured in liquid broth and incubated at 28°C with shaking at 150 rpm for 21 days. On days 7, 14 and 21 sample were collected and tested for antimicrobial susceptibility. The culture was centrifuged at 1,500 rpm for 15minutes; the supernatant was discarded, while the deposit 1,000µl was aliquoted into the agar well against Salmonella sp, Staphylococcus aureus and E. coli. The culture conditions (e.g., temperature, incubation time) were optimised to enhance antibiotic production.

3. Results

Table 1 showed the morphological and biochemical characteristics of actinomycetes isolated from the millet plant rhizosphere. Sample A exhibited a pH of 6.0, and the colonies were white or greyish filamentous with rough textures. The suspected organism was Streptomyces sp. Sample B had a pH of 6.5, and the colonies were golden-yellow and irregular with a powdery texture. In contrast, sample C had a pH of 7.0, with colonies exhibiting circular, yellowish and leathery colonies with Micromonospora spp. As the suspected organism. All isolates were Gram-positive and catalase-positive, confirming their identity as actinomycetes.

Table 2 presents preliminary antibiotic screening results for the isolates against three bacterial species. Streptomyces spp. displayed minimal inhibition against E. coli and Staphylococcus sp., while Nocardia spp. showed minimal inhibition only against E. coli. Neither isolate inhibited Salmonella sp.

Table 3 showed antibiotic production by the isolates over three time points. Streptomyces spp. displayed higher antibiotic activity, particularly on Day 14, with inhibition zones of 25 mm and 30 mm for E. coli and Salmonella sp., respectively, and no activity against Staphylococcus sp. Nocardia spp. demonstrated moderate activity only on Day 12, with 19 mm and 16 mm inhibition zones for E. coli and Salmonella sp., respectively, and no activity against Staphylococcus sp.

Table 1: Morphological Characteristics and Biochemical Test

Sample	PH	Colour	Shape	Texture	Gram Stain	Catalase Test	Suspected Actinomycetes
Millet rhizosphere(A)	6.0	White/Grayish	Filamentous	Rough	+	+	*Streptomyces* spp.
Millet rhizosphere (B)	6.5	Golden Yellow	Irregular	Powdery	+	+	*Nocardia* spp.
Millet rhizosphere (C)	7.0	Yellow	Circular	Leathery	+	+	*Micromonospora* spp.

Table 2: Preliminary Antibiotic Screening

Isolates	*Salmonella* Sp	*Staphylococcus* Sp	*E. coli*
A	No Inhibition	Minimal Inhibition	Minimal Inhibition
B	No Inhibition	No Inhibition	Minimal Inhibition
C	Minimal Inhibition	No Inhibition	No Inhibition

Table 3: Antibiotics susceptibility profile of Actinomycetes producing antibiotics against some bacteria

Actinomycetes Isolates	Time (Days)	Test bacteria with zones of inhibition (mm)		
		E. coli	*Salmonella* Sp	*Staphylococcus* aureus
***Streptomyces* sp**	7	10	15	0.00
	14	30	35	0.00
	21	35	50	20
***Nocardia* sp**	7	0.00	0.00	0.00
	14	20	19	0.00
	21	25	45	25
***Micromonospora* spp.**	7	0.00	0.00	0.00
	14	20	30	22
	21	42	35	40
Control(*Streptomycin*)		29	35	20

4. Discussion

The isolation and screening of antibiotic-producing actinomycetes from the rhizosphere of millet was carried out. The results of the preliminary antibiotic screening reveal varying degrees of antimicrobial activity, with Streptomyces sp. and Micromonospora sp. showing minimal inhibition of E. coli and Salmonella, while Nocardia spp. exhibited limited or no inhibition. This finding agrees with studies by Chater (2016) and Tiwari and Gupta (2012), who recognised Streptomyces sp. for its ability to produce antibiotics, including streptomycin and tetracycline. On the contrary, Nocardia sp. has been reported to show weaker or inconsistent antimicrobial activity in comparison to Streptomyces, possibly due to lower secondary metabolite production as reported by Goodfellow et al. (2012). The minimal inhibitory activity of Micromonospora spp. against Salmonella coincides with its known potential to produce aminoglycosides such as gentamicin, as reported by Davies and Davies (2010).

The isolates exhibited minimal or no inhibitory effect on Staphylococcus, with only Streptomyces spp. showing minimal inhibition on E. coli. This result indicated the specificity of the antimicrobial metabolites produced by the isolated actinomycetes. A study by Hopwood (2007) has shown that Streptomyces and other actinomycetes produce antibiotics with varying spectra of activity, some of which may not be effective against Staphylococcus species.

Streptomyces spp. showed a remarkable increase in antibiotic activity, particularly against E. coli and Salmonella, with inhibition zones reaching 35 mm and 50 mm, respectively, by day 14. This time-dependent increase in antibiotic production agrees with a study by Oskay et al. (2004), who suggested that Streptomyces spp. require extended incubation periods to maximise secondary metabolite production. Bérdy (2012) and Baltz (2017) showed that antibiotic production by Streptomyces often peaks after 10 to 14 days of incubation, during which metabolic processes optimise the synthesis of antimicrobials such as streptomycin and erythromycin.

In the same way, Nocardia spp. showed a sluggish but moderate increase in antibiotic production, with zones of inhibition corresponding to 25 mm against E. coli and 45 mm against Salmonella by day 14. These results coincide with the study by McNeil and Brown (2017), which showed that although Nocardia spp. are less prolific antibiotic producers compared to Streptomyces, they still exhibit the potential to produce antibiotics under certain conditions. In addition, the increase in antibiotic production over time suggests that Nocardia sp. might require longer incubation to achieve measurable activity, a finding which is in consonance with the study by Goodfellow et al. (2012), who highlight the slower metabolic growth of this genus.

The activity of Micromonospora sp. Is remarkable in this study, as it demonstrated potent antibiotic activity by day 14, with zones of inhibition corresponding to 42 mm, 35 mm, and 40 mm against E. coli, Salmonella, and Staphylococcus, respectively. This result coincides with the well-known role of Micromonospora in producing aminoglycoside antibiotics such as gentamicin and neomycin, which are highly effective against a broad range of Gram-positive and Gram-negative bacteria, as reported by Maldonado et al. (2016) and Hong et al. (2015).

The broad-spectrum antibiotic activity observed in this study suggests that Micromonospora sp. could be a promising organism for further investigation, particularly in terms of its potential to produce novel antimicrobial compounds.

5. Conclusion

This study highlights the antibiotic potential of actinomycetes isolated from millet samples, particularly Streptomyces sp. and Micromonospora sp. The results show time-dependent increases in antibiotic activity, particularly by day 14 of incubation. Future research should focus on optimising the conditions for antibiotic production and exploring the potential for novel antimicrobial compounds, especially in the case of Nocardia sp., which demonstrated limited but promising activity. These findings contribute to the growing body of knowledge on the diversity and utility of actinomycetes in antibiotic production.

6. Recommendations

The following recommendations are made from the results of this study:

1. The incubation conditions of the actinomycete species should be optimised for maximum antibiotic production.
2. The Potential of Micromonospora spp. for Broad-Spectrum antibiotic production should be further explored.
3. Nocardia sp. Should be investigated for secondary metabolites, with a focus on enhancing the production of secondary metabolites, which may result in stronger and more diverse antimicrobial properties.

References

Baltz, R. H. (2017). Natural product discovery from actinomycetes: The evolution of new antibiotics. Research in Microbiology, 168(9–10), 718–728.

Bérdy, J. (2012). Thoughts and facts about antibiotics: Where we are now and where we are heading. Journal of Antibiotics, 65(8), 385–395.

Brown-Elliott, B. A., Brown, J. M., Conville, P. S., & Wallace, R. J. (2016). Clinical and laboratory features of the Nocardia spp. based on current and molecular taxonomy. Clinical Microbiology Reviews, 19(2), 259–282.

Chater, K. F. (2016). Recent advances in understanding Streptomyces. F1000Research, 5, 2795.

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews, 74(3), 417–433.

Fiedler, H.-P., Bruntner, C., & Bull, A. T. (2018). Isolation and characterisation of actinomycetes from marine sediments. FEMS Microbiology Letters, 171(1), 11–16.

Goodfellow, M., Kämpfer, P., Busse, H.-J., Trujillo, M. E., Suzuki, K., Ludwig, W., & Whitman, W. B. (2012). Bergey's manual of systematic bacteriology (Vol. 5). Springer.

Hong, H.-J., Paget, M. S., & Buttner, M. J. (2015). Antibiotic production in Streptomyces. Trends in Microbiology, 13(1), 31–37.

Kämpfer, P. (2012). The family Streptomycetaceae, part of the order Actinomycetales. In D. Krieg (Ed.), Bergey's manual of systematic bacteriology (Vol. 5, pp. 148–180). Springer.

Kavitha, A., Prabhakar, P., Narasimha, G., Vijayalakshmi, M., Venkateswarlu, Y., & Rao, K. V. (2010). Isolation, characterisation, and biological evaluation of bioactive metabolites from Streptomyces cheonanensis VUK-A. Microbial Pathogenesis, 49(6), 311–318.

Lechevalier, H. A., & Lechevalier, M. P. (2019). The family Streptomycetaceae. In Bergey's manual of systematic bacteriology (Vol. 4).