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Diversity, abundance, and enzyme activity of Actinomycetes recovered from tropical freshwater sediment for potential biotechnological applications

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Abstract. *Ajuzieogu CA, Onu GO, Onyekachi EO. 2025. Diversity, abundance, and enzyme activity of Actinomycetes recovered from tropical freshwater sediment for potential biotechnological applications. Asian J Trop Biotechnol 22: 23-29.* Actinomycetes are a set of Gram-positive bacteria known for their ability to produce a wide variety of secondary metabolites, including antibiotics and enzymes. This study assessed the diversity of Actinomycetes in tropical freshwater sediment and their amylase production potential. Sediment samples were obtained from four points (shore, middle, downstream, and upstream) within a tropical freshwater system. Physicochemical analysis, bacterial isolation, and enumeration were conducted following standard methods. Tentative identification of isolates was done using culture-dependent methods. Results showed that the sediment samples belonged to the loam soil textural class; upstream sediment recorded the highest pH and total organic carbon content (6.3 and 0.98%, respectively), and middle sediment recorded the highest nitrate content (9.632 mg/kg). In comparison, sediment from the shore recorded the lowest pH (5.70) and highest phosphate level (8.716 mg/kg). The highest total culturable heterotrophic counts were observed in sediment from the middle point. A total of 9 Actinomycetes species were recovered, out of which six (6) were positive for amylase activity. The isolates were tentatively identified as *Micrococcus* spp., *Streptomyces* sp., *Streptomyces coelicolor,* and *Actinomyces viscosus.* This study revealed that tropical freshwater sediments harbor a diverse and abundant bacterial community, particularly Actinomycetes, with possible potential for biotechnological exploration.

Keywords: Actinomycetes, amylase, API CORYNE, freshwater sediment, *Streptomyces coelicolor*

INTRODUCTION

Actinomycetes are Gram-positive bacterial species belonging to the Phylum Actinobacteria. Their DNA has a high Guanine-Cytosine (GC) content, up to more than 70% in *Streptomyces* and *Frankia* and as high as 51% in certain Corynebacteria. Recently, some freshwater-dwelling species with considerably low GC content have been reported (De Simeis and Serra 2021). The majority form branching mycelia and produce spores, which help them survive under diverse environmental conditions (Kontro et al. 2022).

Their ability to produce a myriad of biologically active compounds has made them impactful in medicine and biotechnology. The biotechnological significance of Actinomycetes was first discovered in the 1940s with Selman Waksman's uncovering of streptomycin. This was the first antibiotic effective against tuberculosis (Ahmad et al. 2019). This breakthrough led to more exploration of Actinomycetes for other biologically active compounds. Currently, over two-thirds of natural antibiotics are recovered from Actinomycetes, particularly from the genus *Streptomyces* (Law et al. 2019).

The use of enzymes, particularly of Actinomycetes origin, in food production and other industrial processes is fast advancing to fulfill the demands of the textile, food processing, and pharmaceutical industries. Amylase, which is one of the enzymes produced by Actinomycetes, is one of the frequently exploited enzymes in several starch industries and has several industrial applications in the manufacturing of syrup, re-sizing clothing fabrics, detergent formulations to enhance cleaning performance (i.e., the alkaliphilic Actinomycetes strains), and others. Amylase represents about 25% of the global enzyme market demand (Praveen Kumar et al. 2015; Miao et al. 2018; Shigeri et al. 2019).

Increasing concern about contamination of aquatic and soil ecosystems has led to several studies on the use of Actinobacteria for bioremediation purposes (Alvarez et al. 2017). Although there are extant techniques to remediate contaminated ecosystems, they are not completely successful, especially with respect to inorganic compounds (John et al. 2022). Oftentimes, these compounds are only partially degraded, generating metabolites (intermediates) that could be more harmful than the parent compound. Owing to the presence of Actinomycetes in soil and water and their functional capacity to maintain ecological balance (i.e., breaking down inorganic and organic compounds in their habitat), they are an excellent option for bioremediation. Jagannathan et al. (2021) reported that several *Streptomyces* strains, including *Streptomyces espinosus*, produce tyrosinase enzymes, which are important for eliminating phenols (a common ingredient in pesticides and petroleum products) that contaminate water supplies.

Compared to widely used tyrosinase, which was previously isolated from mushrooms, *Streptomyces*-derived tyrosinase was more effective (Roy et al. 2014).

According to past literature, the majority of Actinomycetes are recovered from soil, aquatic, and extreme ecosystems such as deep oceans and deserts (Ezeobiora et al. 2022), resulting in several bioactive compounds such as anticancer agents, antifungals, antibiotics, enzymes, and other therapeutically valuable compounds (Sarkar and Suthindhiran 2022). However, as interest in microbial diversity has expanded, researchers have increasingly switched focus to less explored environments, including aquatic ecosystems such as freshwater sediments (Selim et al. 2021).

Zothanpuia et al. (2018) highlighted the significance of freshwater sediments as untapped reservoirs of diverse microbial communities, including Actinomycetes. They identified a plethora of Actinomycetes species in freshwater sediments collected from various water bodies in India. Interestingly, many of the recovered strains expressed unique metabolic capabilities and potential for producing novel bioactive compounds with enzyme and antimicrobial activities. Their study unveiled the significance of exploring the unexploited diversity of Actinomycetes in aquatic ecosystems and the vast biotechnological potential awaiting discovery.

Similarly, Sarkar and Suthindhiran (2022) reviewed the diversity and biotechnological potential of Actinomycetes isolated from different marine environments. Their study revealed the presence of novel Actinomycetes species, some of which exhibited promising antimicrobial and enzyme-producing potential. Their study underscored the value of exploring indigenous microbial resources for biotechnological applications.

Despite these notable contributions, there remains a significant gap in our understanding of Actinomycetes diversity in tropical freshwater sediments, especially in regions like Niger Delta, Nigeria. Most research in this area has focused on temperate or subtropical regions, neglecting the unique environmental conditions and microbial communities present in tropical ecosystems (Olanrewaju and Babalola 2018).

Hence, this research aimed to assess the diversity of Actinomycetes in tropical freshwater sediments and their enzyme (amylase) production potential for biotechnological exploration.

MATERIALS AND METHODS

Study area

This study was conducted at the Department of Microbiology, Federal University Otuoke, Bayelsa State, Nigeria, which is situated at coordinates: Latitude 4°.47'27.91" N and 4°.47'44.37" N of the Equator and Longitude 6°19'19.4" and 6°19'52.12" E of the Greenwich Meridian.

Sample collection

Freshwater sediments were collected from Otuoke River, in Ogbia Local Government Area, Bayelsa State, Nigeria, at coordinates: Latitude 4°.57'16.83" N and 6°.18'35.87" E. The samples were collected from four different points 50 meters apart (i.e., upstream, middle, downstream, and shore) in Otuoke River, Bayelsa State. The sediment samples were collected from each point at a depth between 0-15 cm using a grab sampler, placed in sterile glass containers, and transferred into an ice pack (Song et al. 2023). These were transported to the laboratory for further analysis.

Physio-chemical analysis

The conductivity and pH were measured using a HANNA Conductivity meter (HI 9835) and a digital Oakton pH meter (model PCD 650), respectively. The pH and conductivity meters were standardized with buffer solutions (4 & 7) and conductivity standard solutions, respectively. The tip of the probe in each case was rinsed with deionized water and cleaned with a paper towel. The probe was then immersed in the sample, and the corresponding steady reading was taken in each case. Nitrate, phosphate, and total organic carbon were determined following the method adopted by APHA (2017).

Determination of nitrate

This was done following the method adopted from (APHA 4500-NO_3-B). Next, a sample cell was filled with 10 mL of the sample, and the contents of one Nitra Ver 5 Nitrate Reagent powder pillow were added (the prepared sample). A second sample cell was filled with the sample (the blank) used in zeroing the equipment. The prepared sample was then placed into the cell holder and resulting in parts per million (ppm) nitrate nitrogen (NO_3-N) being read. The equipment used was a DR 5000TM UV spectrophotometer.

Total Organic Carbon (TOC)

The TOC method was adopted from APHA 5310B (high-temperature combustion method). The sample is homogenized, and a micro portion is injected into the reaction chamber packed with heated barium chromate. The water was then vaporized, and the organic carbon was oxidized to H_2O and CO_2. The CO_2 from the oxidation of inorganic and organic carbon is taken in the carrier gas stream and measured by means of an infrared analyzer.

Phosphate determination

The method for phosphate determination was adopted from APHA 4500-P. Next, 10 mL of the sample was added to the sample cell, and the content of one Phosphate Reagent Powder Pillow was added (the prepared sample). A second sample cell was filled with the sample (the blank) used in zeroing the equipment. The prepared sample was then placed into the cell holder, and the result in ppm was read. The equipment used was a DR 5000TM UV spectrophotometer.

Isolation and enumeration of bacteria

Isolation and enumeration were done according to the method of Adeyemo et al. (2021). Freshwater sediment samples were serially diluted up to $10^{-5.}$ Then, a 0.1 mL

aliquot of each dilution (10^{-4} and 10^{-5}) was inoculated using the spread plate method on starch casein agar and nutrient agar plates in duplicates. The plates were incubated for 5 days at 37±2°C. After incubation, the morphological characteristics of the colonies were examined and recorded, and all discrete colonies were counted and expressed in colony-forming units per gram (CFU/g). Different colonies were subcultured on starch casein agar and later incubated for another 48 hours at 37°C to obtain pure cultures, which were preserved on nutrient agar slants at 4±2°C for further analyses.

Screening of Actinomycetes for amylase activity

Isolates were screened for amylolytic properties by starch hydrolysis test on starch agar plates following the procedure described by Praveen Kumar et al. (2015). The streaked isolates were incubated at 30±2°C for 7 days. After the incubation, 1% iodine solution (freshly prepared) was flooded on the starch agar plates, and a clear zone of hydrolysis was considered as amylase producers.

Biochemical characterization of the isolates

Gram staining and other biochemical tests, such as catalase, citrate, Triple Sugar Iron (TSI), motility, indole, and urease (MIU), were performed on the isolates following the standard procedures of Cheesbrough (2010).

Gram staining

A thin smear of the bacterial culture was prepared on a clean, grease-free glass slide and allowed to air dry. The smear was heat-fixed by passing the slide through the flame. The smear was flooded with crystal violet for 60 seconds before rinsing gently with distilled water. Lugol's iodine solution was added and allowed to sit for 60 seconds before rinsing gently with distilled water. The smear was decolorized with 95% ethanol for 30 seconds before rinsing with distilled water. It was then counter-stained with safranin for 60 seconds before rinsing with distilled water and air dried. Gram-stained slides were examined under a microscope with a ×100 objective lens using oil immersion.

Catalase test

A drop of aqueous hydrogen peroxide solution was placed on a grease-free slide, and a loopful of the test organism was smeared on it. The mixture on the slide was observed for the production of gas bubbles, which indicated a positive reaction result.

Citrate utilization test

This test determined the ability of bacteria to use citrate as their sole carbon source. Each isolate was inoculated on a freshly prepared Simmons' citrate agar slant and incubated at 37°C for 12-24 hours. A change of color from blue to green indicated a positive result, while no color change indicated a negative result.

Triple Sugar Iron (TSI) agar test

This test was carried out to differentiate bacteria based on their ability to ferment sugars (glucose, lactose, and sucrose) and produce hydrogen sulfide. During the test, the butt of the TSI agar slant was stabbed with an inoculating needle containing the test organism, then streaked on the surface of the slant. The slant was incubated at 37°C for 18-24 hours. After which, it was observed for color change and gas production. The result was interpreted as follows: yellow slant/yellow butt: glucose, lactose, and/or sucrose fermentation; red slant/yellow butt: glucose fermentation only; red slant/red butt: no sugar fermentation; black precipitate in the butt: hydrogen sulfide production; cracks or bubbles: gas production.

Motility-Indole-Urea (MIU) test

This test was carried out to determine bacterial motility, indole production, and urease activity in a single test. Next, using a sterile inoculating needle, the MIU medium was stabbed with the test organism. The test tube was incubated for 24-48 hours at 37°C. Next, the test tubes were observed for motility by checking for diffusion of growth away from the stab line after the incubation period. A few drops of Kovac's reagent were added to the medium to test for indole production. The tube was also observed for a color change in the medium, indicating urease activity. The results were interpreted as follows: Motility is positive if there is a diffuse growth spreading away from the stab line and negative if growth is only along the stab line.

Indole production was considered positive if a red or pink color appeared after adding Kovac's reagent, and negative if there was no color change. Urease production was considered positive if the medium turned pink, and negative if there was no color change.

Identification of the isolates using the (Analytical Profile Index (API) CORYNE, BioMerieux, France) test kit

The API CORYNE strip consists of 20 microtubes containing dehydrated substrates for the demonstration of enzymatic activity or the fermentation of carbohydrates (CHO). The addition of a dense test suspension of bacteria rehydrates the enzymatic substrates. The metabolic end products produced during incubation are detected through spontaneous colored reactions or by the addition of reagents. The fermentation tests are inoculated with an enrichment medium (containing a pH indicator), which reconstitutes the CHO substrates. Color changes in the pH indicator detect fermentation of CHO.

To carry out the fermentation tests, about 0.5 mL of bacterial suspension was transferred to an ampoule containing 2 mL of GP medium. After homogenization, this new suspension was distributed into the fermentation tubes and overlaid the cupules with mineral oil. The same was done for the urea hydrolysis tube. The strip was then incubated at 37°C for 24 hours. Blood agar was also incubated as a control.

The readings, except for the esculin, urease, and gelatin tests, were done after adding the appropriate reagents. The fermentation reactions were considered positive when they turned yellow.

The reactions are read according to the Reading Table and the identification is obtained by referring to the Analytical Profile Index or using the APIweb, API identification software.

RESULTS AND DISCUSSION

Physico-chemical characteristics

The physico-chemical properties of the sediment samples are presented in Table 1. Upstream sediment recorded the highest pH and TOC values (6.30 and 0.98%), while shore sediment recorded the lowest pH (5.70) and the highest phosphate concentration (8.716 mg/kg). Sediment from the middle recorded the highest nitrate (9.632 mg/kg) and conductivity (115 µs).

Bacterial counts from sediment samples across different microbiological media

The results revealed that middle sediment recorded the highest total culturable heterotrophic bacterial counts (TNTC) on Nutrient agar, followed by upstream sediment (1.46×10^{-7}CFU/g). At the same time, sediments from downstream and shore were too few to count (TFTC), as shown in Table 2.

Enzyme (amylase) activity of isolates

Table 3 presents the enzyme activities of the isolates. Six (6) isolates were positive for amylase production (Figure 1).

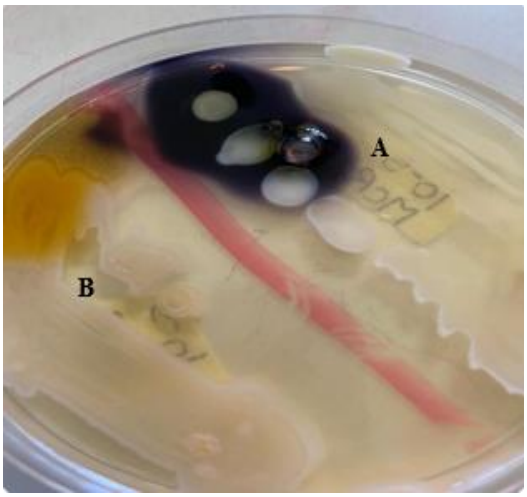

Figure 1. A. Positive Amylase activity of *Micrococcus* sp. (SMCR2); B. Negative amylase activity of *Streptomyces* sp. (SD C)

Biochemical characteristics and analytical index profile (API) identities of isolates

The biochemical characteristics and API tests tentatively identified the isolates as *Micrococcus roseus*, *Micrococcus luteus* (Schroeter, 1872) Cohn, 1872, *Micrococcus* sp., *Actinomyces* sp., *Actinomyces viscosus* (Howell et al., 1965) Georg et al., 1969, *Streptomyces coelicolor*, and *Streptomyces* sp. (Tables 4 and 5).

Table 2. Enumeration of bacterial isolates on various microbiological media

Sample code	Total culturable heterotrophic bacteria counts (CFU/g) on NA	Actinomycetes count (CFU/g) on SCA
SS	TFTC	3.4×10^{-7}
SM	TNTC	6.2×10^{-7}
SD	TFTC	1.6×10^{-7}
SU	1.46×10^{-7}	TFTC

Note: NA: Nutrient agar; SCA: Starch casein agar; TFTC: Too few to count (less than 30 colonies); TNTC: Too numerous to count (greater than 30 colonies and above); SS: sediment from shore; SM: Sediment from middle; SD: Sediment from downstream; SU: Sediment from upstream, CFU/g: Colony forming unit per gram

Table 3. Amylase activity of the isolates

Isolate code	Amylase activity
SM CR2	+
SM CR	-
SS WR	+
SD C	-
SM CI	+
SD CM	-
SM CE	+
SS W	+
SM Y	+

Note: +: positive; -ve: negative

Table 1. Physicochemical characteristics of sediment samples

Parameters	Analysis method	SU	SM	SD	SS
Phosphate (mg/kg)	APHA 4500-P	2.404	2.152	2.132	8.716
Nitrate (mg/kg)	ASTM D3867-04	6.624	9.632	3.600	2.488
pH	ASTM D1293-99	6.30	6.10	6.20	5.70
Conductivity (µs)	ASTM D 1125	107	115	95	101
TOC (%)	APHA 5310B	0.98	0.86	0.69	0.80
	PSD				
Sand (%)	Sieve	35.30	28.20	37.70	27.70
Clay (%)	Sieve	5.50	7.90	4.20	8.30
Silt (%)	Sieve	59.10	61.40	57.30	62.00

Note: SS: Sediment from shore; SM: Sediment from the middle; SD: Sediment from downstream; SU: Sediment from upstream; PSD: Particle size distribution

Table 4. Biochemical characterization of the bacterial isolates

Isolate code	Shape	Color	Citrate	Catalase	Slant	TSI Butt	Gas	H_2S	Motility	MIU Indole	Urease	Tentative identity of organism
SM CR2	Cocci	Purple	-ve	+ve	Yellow	Red	-ve	-ve	+ve	-ve	+ve	*Micrococcus* sp.
SM CR	Rods	Purple	+ve	+ve	Red	Yellow	-ve	-ve	-ve	+ve	-ve	*Streptomyces* sp.
SS WR	Rods in diploid	Purple	-ve	+ve	Yellow	Red	-ve	-ve	+ve	-ve	+ve	*Streptomyces* sp.
SD C	Filamentous rods with spores	Purple	-ve	+ve	Yellow	Yellow	-ve	-ve	-ve	-ve	+ve	*Streptomyces* sp.
SM CI	Cocci	Purple	-ve	+ve	Red	Red	-ve	-ve	-ve	-ve	+ve	*Micrococcus* sp.
SD CM	Filamentous rods	Purple	-ve	+ve	Red	Red	-ve	-ve	-ve	-ve	+ve	*Actinomyces* sp.
SM CE	Cocci	Purple	-ve	+ve	Red	Yellow	+ve	-ve	+ve	-ve	+ve	*Micrococcus* sp.
SS W	Cocci in clusters	Purple	-ve	+ve	Yellow	Yellow	-ve	-ve	+ve	-ve	+ve	*Micrococcus roseus*
SM Y	Cocci	Purple	-ve	+ve	Red	Yellow	-ve	-ve	-ve	-ve	+ve	*Micrococcus luteus*

Note: +ve: Positive; -ve: Negative; TSI: Triple Sugar Iron (Cheesbrough 2010)

Table 5. Identification of bacterial isolates using the Analytical Profile Index test

Isolate code	IND	URE	GLU	MAN	LAC	SAC	MAL	SAL	XYL	ARA	GEL	ESC	GLY	CEL	MNE	MLZ	RAF	SOR	RHA	TRE	CAT	API % SIMILARITY IDENTITY	ORGANISM TENTATIVE IDENTITY
SS WR	-	+	-	+	+	-	NT	NT	-	+	+	+	-	-	-	-	+	-	+	-	+	99.9%	*Streptomyces coelicolor*
SD CM	-	-	+	-	+	+	+	-	-	-	-	-	+	-	+	-	+	-	-	-	+	98.4%	*Actinomyces viscosus*

Note: +: Positive; -: Negative; IND: Indole; URE: Urease; GLU: Glucose; MAN: Mannitol; LAC: Lactose; SAC: Saccharose; MAL: Maltose; SAL: Salicin; XYL: Xylose; ARA: Arabinose; GEL: Gelatin; ESC: Esculin; GLY: Glycerol; CEL: Celliobiose; MNE: Mannose; MLZ: Melezitose; RAF: Raffinose; SOR: Sorbitol; RHA: Rhamnose; TRE: Trehalose; CAT: Catalase; NT: Not tested (Soto et al. 1994)

Discussion

The variations observed in phosphate, nitrate, pH, conductivity, and TOC across different sediment locations indicate diverse microenvironments that influence microbial diversity. Nitrate concentrations were notably higher in sediment from the middle, suggesting that this area may have a higher level of nutrient influx or retention compared to the shore and downstream sediments. The sediments from the shore and middle had the highest phosphate and nitrate content levels, respectively (Table 1), which are conducive for microbial growth (high bacterial population), as shown in Table 2, supporting previous studies that link higher nutrient levels with increased microbial activity (Francioli et al. 2016; Maron et al. 2018). On the other hand, the lower pH observed in shore sediment could be attributed to a higher accumulation of organic matter, such as decaying plant material and other organic debris around shore areas, which usually undergo microbial decomposition, resulting in the production of organic acids such as humic and fulvic acids, which can lower the pH of the sediment alongside other ongoing anthropogenic activities (Lalas et al. 2018; Yang et al. 2019).

The particle size distribution results showed that silt was the dominant fraction in all samples, which is consistent with findings by Xia et al. (2020), who stated that fine particles like silt provide a greater surface area for microbial colonization and are usually associated with the relative abundances of some fungi and filamentous bacteria such as Actinobacteria. The combination of these physicochemical properties possibly contributed to the microbial diversity and abundance observed in the sediments.

The diversity, abundance, and amylase activity of isolates recovered in this study emphasize the rich microbial ecosystem within these sediments, highlighting their potential as a source of novel bioactive compounds with significant biotechnological value (Praveen Kumar et al. 2015; Zothanpuia et al. 2018; Shigeri et al. 2019; Ribeiro et al. 2023).

The bacterial counts from the sediment samples revealed significant variations in microbial abundance, especially between different points and media types. The highest count was observed in the middle sediment on SCA, which aligns with the nutrient-rich nature of this location as indicated by its higher nitrate content (Table 2). This finding is particularly enlightening as it is consistent with the findings of Li et al. (2023), who reported that microbial biomass and composition in sediment were strongly influenced by nitrogen inundation (nutrient availability). The lower microbial population observed in downstream sediment suggests a less favorable environment for heterotrophic bacteria and Actinomycetes or starch-degrading bacteria, possibly due to lower organic carbon, nitrate, and phosphate content (Tables 1 and 2). Similar observations were reported by Zanane et al. (2018), where the presence of Actinomycetes recovered in their study was majorly influenced by the prevailing physicochemical properties (e.g., organic carbon, pH, moisture, and others).

The biochemical characterization and API tentative identification of the isolates further emphasize the diversity of the bacterial community within the sediment samples. The prevalence of *Micrococcus* and *Streptomyces* species is notable, as these genera are well-known for their resilience in diverse environments and their ability to produce antibiotics, enzymes, and other secondary metabolites (Barka et al. 2016). The identification of *S. coelicolor* with a 99.9% similarity identity is consistent with previous studies that recovered this species from diverse soil and sediment environments (Patin et al. 2017). *Streptomyces* species are renowned for their secondary metabolite production, including antibiotics, which highlights the potential biotechnological applications of these isolates (Alam et al. 2022). The presence of *Actinomyces viscosus*, known for its role in soil health and organic matter decomposition, further supports the rich microbial diversity observed in these sediments (Ataikiru et al. 2020). The overall identification results are consistent with those of Ribeiro et al. (2020), who reported similar bacterial diversity in tropical sediments, with a significant presence of Actinomycetes and other gram-positive bacteria.

In conclusion, this study highlights the rich bacterial diversity and abundance present in tropical freshwater sediments, especially among Actinomycetes, which were found to be abundant and diverse across the different sediment locations within the freshwater system. The variations in physicochemical properties, especially pH, nutrient content, and particle size distribution, played a significant role in shaping the bacterial community. The identification of species with known biotechnological potential (amylase activity), such as *S. coelicolor* and *M. luteus*, emphasizes the value of these sediments as sources of novel bioactive compounds. These findings provide a foundation for future research aimed at exploring the biotechnological applications of these microorganisms, particularly in the development of new antibiotics, enzymes, and other secondary metabolites, and for their molecular identification, as this gives a more accurate identification of microorganisms compared to culture-dependent identification methods used in this study.

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