ORIGINAL ARTICLE

Antimicrobial Activities of Actinomycetes Isolated From Flooded and Unflooded Soils

Koh Hui Yi[1], Nor Farahani Ali Othman[1], Ainihayati Abdul Rahim[2], Seri Intan Mokhtar[1], *Khomaizon Abdul Kadir Pahirulzaman[1]

[1] Faculty of AgroBased Industry, Universiti Malaysia Kelantan, Jeli campus, 17600 Jeli, Kelantan, Malaysia.
[2] Faculty of Bioengineering and Technology, Universiti Malaysia Kelantan, Jeli campus, 17600 Jeli, Kelantan, Malaysia.

ABSTRACT

Introduction: Flooding may cause the microbial population in the soil to move from one area to another. Actinomycete, a type of soil microbe, has the most commercial value due to its ability to produce secondary metabolites. This study aimed to elucidate the antimicrobial activities of actinomycetes isolated from flooded and unflooded areas. **Methods:** Soils samples were collected from flooded areas in Dabong, Kelantan, and unflooded areas in Jeli, Kelantan. Three isolation methods were used to isolate actinomycetes; Sonication, Centrifugation and Chloramine T. The isolated strains were screened for morphological characteristics based on their growth pattern (spore formation), colony color, aerial and substrate mycelia color, and soluble pigment formation in the growth medium. Morphologically different strains were tested against *Escherichia coli* and *Candida albicans* for its antibacterial and antifungal activities. **Results:** A total of 970 actinomycete strains were isolated from soil samples (570 strains from flooded soil and 400 strains from unflooded soils). Only 281 strains were morphologically different. Thirty actinomycete strains were tested for antibacterial and antifungal activity. Seventeen of these inhibit at least one test microorganism. **Conclusion:** In conclusion, our observations reveal that the soil samples obtained from flooded areas display a wide variety of actinomycetes, as evident from their morphological characteristics. This finding suggests that the flooded soil areas possess a higher diversity of actinomycetes compared to non-flooded soil areas. Furthermore, we found that 57% of the tested actinomycete strains exhibited activity against at least one test organism, indicating their potential for future research.
Malaysian Journal of Medicine and Health Sciences (2023) 19(SUPP9): 42-49. doi:10.47836/mjmhs.19.s9.7

Keywords: Actinomycetes; Flood; Antimicrobial assay; Secondary metabolites

Corresponding Author:
Khomaizon Abdul Kadir Pahirulzaman, PhD
Email: khomaizon@umk.edu.my
Tel: +60129715942

INTRODUCTION

Aquatic environments differ considerably in chemical and physical properties, and it is not surprising that their microbial species compositions also differ. Microorganisms' natural habitats are incredibly diverse. The growth of microorganisms can occur in any environment that is suitable for the development of higher organisms. Furthermore, there are many habitats where higher organisms are absent due to some physical or chemical extreme, but microorganisms exist and occasionally flourish. In the consideration of flooded areas, our attention inevitably turns to soil because the most extensive microbial growth takes place on the surfaces of soil particles, usually within the rhizosphere. One of the major factors affecting microbial activity in soil is the availability of water, which has variety of material dissolved in it. Bacteria, specifically actinomycete, a Gram-positive bacteria that belong to the Actinomycetales order within the Actinobacteria phylum and include a wide range of members with significant medical and economic importance. For example, filamentous actinomycetes, such as *Streptomyces* species, produce a plethora of bioactive secondary metabolites and form symbiotic relationships with a variety of organisms, including plants and insects. Investigating these bacteria is both difficult and fascinating, but it can also yield substantial rewards (1). Actinomycetes are a significant potential source of new bioactive substances due to their diverse metabolite characteristics and advanced metabolic differentiation. It is capable of producing a wide range of biologically active secondary metabolites (2) and responsible for approximately 80% of the world's antibiotics, most of which are derived from the genera *Streptomyces* and *Micromonospora*; the remainder are derived from filamentous fungi

and non-actinomycete bacteria. (3, 4, 5). The genus *Streptomyces* has the highest abundance of actinomycetes in soil, accounting for 50% of the total population, and is known for producing a wide range of bioactive secondary metabolites for pharmaceuticals and agricultural use (6, 7). The high biodiversity of actinomycetes has led to the discovery of numerous novel secondary metabolites (8, 9), and their isolation from various environments has been the focus of numerous studies. For example, a recent review by Selim et al., 2021 (10) highlights the importance of studying the secondary metabolites and biodiversity of actinomycetes, particularly in the context of drug discovery. Despite their significance, actinomycetes remain underexplored, and there is still much to be learned about their diversity and potential applications. In this study, we focus on the isolation and analysis of actinomycetes from flooded and non-flooded areas, with the aim of identifying potential sources of antimicrobial agents.

Antimicrobial resistance is a growing global health threat, with the potential to cause significant morbidity and mortality worldwide (11). The development of new antimicrobial agents is essential for combating resistant strains of bacteria, viruses, and fungi, and for addressing the issue of antibiotic overuse (12). Antimicrobial analysis is crucial for understanding the mechanisms underlying resistance and for identifying new treatment strategies (13). It can also provide valuable information about the safety and efficacy of existing antimicrobial agents, guiding clinical decision-making and improving patient outcomes (14). In this study, we used *Candida albicans* and *Escherichia coli* in studying the activity of antimicrobial agents in actinomycetes, which can ultimately lead to the development of new treatments for infectious diseases caused by these and other pathogens.

C. albicans is an important fungus commonly found in the human body, especially in the mouth, gastrointestinal tract, and female genital tract (15). While it is typically harmless, under certain conditions, it can cause infections, especially in immunocompromised individuals (16). Its significance in antimicrobial assay lies in its ability to serve as a model organism for testing the efficacy of antimicrobial agents (17). Due to its widespread prevalence and potential for causing infections, *Candida* is an important target for developing new antimicrobial agents (18). In addition, its ability to form biofilms, which are highly resistant to antimicrobial agents, makes it an excellent candidate for evaluating the activity of potential biofilm-disrupting compounds (19).

E. coli is a gram-negative bacterium commonly used as a model organism in antimicrobial analysis. It is well characterized and has been extensively studied, making it a useful model organism for understanding the molecular mechanisms of antimicrobial resistance (20). *E. coli* infections are a significant cause of morbidity and mortality worldwide, particularly in vulnerable populations such as children and the elderly (21). Antimicrobial resistance in *E. coli* can make infections more difficult to treat, underscoring the need for effective antimicrobial agents (13). Additionally, *E. coli* is commonly used in antimicrobial susceptibility testing, an important tool for guiding clinical decision-making and monitoring resistance patterns (22). Thus, the current study was conducted to investigate the antimicrobial properties of actinomycetes isolated from soil samples collected from both flooded and unflooded areas.

MATERIALS AND METHODS

Sample collection and pre-treatment process

In this study, we collected 10 soil samples, with five samples obtained from a flooded area in a village along the riverside of Dabong, Kelantan, while the other five samples were collected from an unflooded area at Universiti Malaysia Kelantan, Jeli, Kelantan, which served as the control group for the experiment. The soil samples were collected from a depth of 10 cm below the soil surface and were subsequently placed in zip-lock plastic bags. Afterward, the soil samples were air-dried in the laboratory for one week and then stored at 4 °C for future use.

Isolation of actinomycetes

With some modifications, we used three isolation techniques from Muramatsu et al., 2009 (23):

i) Sonication

0.2 g of air-dried soils were mixed with 2 mL of sterilized 0.01% polyoxyethylene sorbitan monoelate, vigorously vortexed for 5 min and sonicated for 5 min. 200 µL of the suspension was inoculated onto ZSSE media (24) (0.5% soluble starch, 0.1% potassium nitrate (KNO_3), 1% agar, and 98.4% soil extracts solution) supplemented with cycloheximide (50 µg/mL) and trimethoprim (50 µg/mL). The plates were incubated for 7 to 14 days at 30 °C with daily observation.

ii) Centrifugation

0.2 g of air-dried soils were gently mixed with 2 mL of sterilized 0.01% polyoxyethylene sorbitan monoelate and incubated at 30 °C for 1 hr. 1 mL of suspension was then centrifuged at 7000 rpm for 20 min. 200 µL of the supernatant was inoculated onto ZSSE media (24). The plates were incubated for 3 weeks at 30 °C with daily observation.

iii) Chloramine T

0.2 g of air-dried soils were mixed with 2 mL of 4% Chloramine T solution (dissolved in sterile water) and vigorously vortexed for 5 min. Then, mixture

was sonicated for 5 min and incubated at 30 °C for 30 min. 200 µL of the suspension was inoculated onto ZSSE media (24). The plates were incubated for 3 weeks at 30 °C with daily observation.

Morphological Characteristics

Actinomycetes were streaked onto new ZSSE media supplemented with trimethoprim (50 µg/mL) and cycloheximide (50 µg/mL) using sterile toothpick. The plates were incubated for 7 days at 30 °C. All actinomycetes growth were observed and compared to each other through morphological characteristics such as color of substrate mycelium, color of aerial mycelium, absence or presence of pigmentation and spore formation (23). When multiple isolates shared the same morphological features, only one actinomycete strain was kept for future use.

Antimicrobial Assay

The antifungal activities of selected actinomycetes were tested by agar plug method against *C. albicans* (25). Selected actinomycetes were streaked on ZSSE media and incubated at 28 °C for 7 days as a lawn. *C. albicans* were grown on Sabouraud's Dextrose Agar (SDA). Agar plugs were prepared using sterile 10 mm cork borer from actinomycete lawns and transferred onto freshly prepared *C. albicans* lawn. Nystatin and sterile distilled water were used as positive and negative control respectively. Plates were incubated at 30 °C for 24 to 48 hours. Presence or absence of inhibition zone was observed and recorded during the incubation period.

Antibacterial activity of actinomycetes was tested using agar plug method against *Escherichia coli* (26). Selected actinomycetes were streaked on ZSSE media and incubated at 28 °C for 7 days as a lawn. *E. coli* was grown on Nutrient Agar (NA). Agar plug from actinomycete lawn was prepared using sterile 10 mm cork borer and transferred aseptically onto freshly prepared *E. coli* lawn. Trimethoprim and sterile distilled water were used as positive and negative controls, respectively. Plates were incubated at 37 °C for 12 to 24 hours. Presence or absence of inhibition zone was observed and recorded during the incubation period.

RESULTS

Ten soil samples were collected from each of the flooded and unflooded areas in Dabong and Jeli, Kelantan, Malaysia respectively. A total of 970 actinomycete strains were isolated from soil samples (570 strains from flooded soil and 400 strains from unflooded soils) using 3 methods; centrifugation (100 isolates), sonication (540 isolates) and Chloramine T (330 isolates). The isolated strains were screened for morphological characteristics based on their growth pattern (spore formation), colony color, aerial and substrate mycelia color, and soluble pigment formation in the growth medium. Only 281 strains were found to be morphologically different (Table I). Of these, only 10 actinomycete strains isolated from each isolation method were selected for antimicrobial analysis. The morphological characteristics of these strains are shown in Table II. Antimicrobial assays against *E. coli* and *C. albicans* were performed on 30 selected strains. Seventeen strains were able to inhibit the growth of at least one test microorganism (Table III).

DISCUSSION

Floods in Malaysia are considerably common, especially during the monsoon season. Floods can create an environment that is conducive to the survival and spread of microorganisms. Floodwaters can carry a variety of pathogens, including bacteria, viruses, and parasites, that can cause serious illnesses such as diarrheal diseases, respiratory infections, and skin infections. The movement of water during flooding can transport pathogens over long distances, increasing the risk of exposure to a large population. Floodwaters can also contaminate drinking water sources, which can lead to outbreaks of waterborne diseases. However, the crisis has allowed researchers to investigate the possibility of isolating novel actinomycetes from the soil of flooded areas. Flooding caused soil saturation, which led to a reduction in soil oxygen. Because of this, the soil has developed anaerobic conditions, which are unfavorable to microorganisms that typically rely on the soil's oxygen. However, when saturated anaerobic

Table I : Morphologically different actinomycetes isolated from flooded and unflooded soils using three isolation methods

Soil sample	Isolation method		
	Sonication	Centrifugation	Chloramine T
Flooded	96	25	81
Unflooded	38	20	21

Table II : Morphological characteristics of actinomycetes strains selected for antimicrobial assays.

Isolation method	Isolate No.	Color of aerial mycelium	Color of substrate mycelium	Soluble pigment	Spore Formation
Sonication	U014	Cream	Cream	Brown	+
	U015	Pink, purple	Red, brown	Maroon	+
	U020	Orange, white	Orange, cream	Red, brown	+
	U029	Green, white	Green	Green	+
	U037	Pink, white	Pink	Brown	+
	D001	White, grey, blue	Cream, white	ND	+
	D002	Brown	Brown	Brown	+
	D007	Pink, white	Red, orange	Purple	+
	D009	Purple, grey	Brown grey	ND	+
	D013	Pink, white	Pink	ND	+
Centrifugation	U109	Grey	Grey	ND	-
	U120	White	White	Yellow	+
	U122	White, grey	Brown, cream	Brown	+
	U126	Dark grey	Dark grey	Green	+
	U127	White, pink	Cream, white	Brown	+
	U129	Purple, grey	Cream, brown	ND	+
	D102	Green, grey	Pink, white	Pink	+
	D108	Brown, cream	Cream, orange layer	Orange	+
	D112	Pink, white	White	ND	+
	D120	Grey, brown	Cream, brown	ND	+
Chloramine T	U202	Dark grey	Grey	Brown	+
	U209	Cream	Cream, white	Brown	+
	U211	White, pink	Pink, white	Pink	+
	U214	Pink	Maroon, brown	Red, brown	+
	D203	Brown	Yellow, brown	ND	+
	D212	Brown, grey	Brown, grey	ND	+
	D275	White	Green, white	Green	+
	D281	Pink, white	Violet	ND	+
	D285	White	White	ND	+
	D292	Yellow, grey, white	Grey	Green	+

ND: not detected

Table III : Antimicrobial activities of actinomycetes against *E. coli* and *C. albicans*.

Isolation method	Isolate No.	Diameter of inhibition zone against test microorganism (mean ± standard deviation in mm)	
		E. coli	***C. albicans***
Sonication	Positive control	24	11
	Negative control	ND	ND
	U014	ND	14.2 ± 0.06
	U015	ND	ND
	U020	10.6 ± 0.06	ND
	U029	ND	ND
	U037	ND	21.5 ± 0.17
	D001	ND	ND
	D002	ND	11.3 ± 0.15
	D007	ND	13.7 ± 0.10
	D009	ND	ND
	D013	ND	ND
Centrifugation	Positive control	25	9
	Negative control	ND	ND
	U109	9.5 ± 0.06	ND
	U120	9.5 ± 0.15	14.3 ± 0.25
	U122	ND	13.8 ± 0.10
	U126	ND	15.2 ± 0.15
	U127	ND	21.7 ± 0.32
	U129	ND	12.2 ± 0.06
	D102	10.2 ± 0.10	ND
	D108	ND	ND
	D112	10.8 ± 0.12	ND
	D120	ND	ND
Chloramine T	Positive control	19	9
	Negative control	ND	ND
	U202	ND	ND
	U209	14.0 ± 0.10	ND
	U211	22.5 ± 0.06	23.3 ± 0.10
	U214	ND	ND
	D203	ND	ND
	D212	ND	ND
	D275	ND	ND
	D281	ND	15.7 ± 0.10
	D285	ND	ND
	D292	ND	18.8 ± 0.10

ND: not detected.

circumstances occurred, a change in the makeup of the microbial population in flooded soil was expected (27, 28, 29). Among the soil microorganisms, actinomycetes have the highest commercial value and are sources of antibiotics (30). Screening and isolating actinomycete strains with potential antibiotics is a promising approach to discovering new antibiotics. Occasionally, pathogens become resistant to antibiotics, and the search for new antibiotics is an ongoing process to update and improve antibiotic effectiveness (30) constantly. Although numerous antibiotics have been found, only a small number of them are currently used in the production of medication for humans and animals. This is because the majority of them are incredibly toxic. Thus, the pharmaceutical sector needs to find new antibiotics that are more potent and low toxicity. Approximately 80% of the world's antibiotics are known to be produced by actinomycetes, primarily the genera *Streptomyces* and *Micromonospora*, with the remainder produced by filamentous fungi and non-actinomycete bacteria (3, 4, 5). The most abundant actinomycetes in soil are from the genus *Streptomyces*, which accounts for 50% of the total population of soil actinomycetes and is known for producing a wide range of bioactive secondary metabolites for pharmaceutical and agricultural used.

In this study, we used three isolation methods to identify 970 actinomycete strains from 10 different soils, both flooded and unflooded. Of these strains, 58.76% were found in flooded soils. We observed morphologically distinct characteristics in only 281 strains, and 26 (9.3%) were found in both types of soil. Interestingly, a higher percentage of actinomycetes (67.3%) were isolated from flooded soils compared to unflooded soils (23.5%). These strains were identified based on their morphology, and the majority produced soluble pigment. Our findings suggest that the potential for discovering new or uncommon actinomycetes is higher in flooded soils. The unique and adaptable morphology that actinomycetes have made it prevalent and can be isolated in even the most extreme condition. The formation of various spores is the primary mechanism for these actinomycetes to survive in these environments. Actinomycetes spores can be used to discriminate against other Gram-positive bacteria since they are typically resistant to desiccation and heating (31). In response to environmental stress, most actinomycete spores are produced either endogenously or exogenously (32). When given an energy source, these spores might be coaxed to germinate in a specified medium despite typically remaining dormant with minimal respiration (33). To improve the efficiency of the isolation process, we pre-treated the soil samples by air-drying. This method has an advantage as many Actinobacteria produce spores that have low respiration rates and can survive for longer periods of time (34). Sonication proved to be the most effective method, isolating 47.7% of the morphologically distinct actinomycete strains. Chloramine T and centrifugation were used to isolate 36.3% and 16.0% of the strains, respectively. Sonication is known to release actinomycete propagules from sediment particles into suspension and increase the number of Actinobacterial strains while reducing undesirable bacteria (35). While non-filamentous bacteria can grow unhindered by the chlorine-releasing biocide Chloramine T, motile actinomycetes like *Actinoplanes* and *Pseudonocardia* can be isolated using the centrifugation technique (35). Actinomycetes spores are also resistant to various chemicals, including sodium dodecyl sulphate, phenol and various antibiotics. These substances increase the probability of isolating actinomycetes while reducing other microorganisms by killing or inhibiting aerobic Gram-negative bacteria, endospore-forming bacilli, and pseudomonads (36).

Thirty isolated actinomycete strains were tested for antibacterial and antifungal activities. Seventeen (57%) of the 30 actinomycete strains demonstrated antimicrobial activity, inhibiting the growth of at least one test microorganism (*E. coli* or *C. albicans*). Ten actinomycete strains had antifungal activity against *C. albicans*, 5 had antibacterial activity against *E. coli*, and 2 had both antifungal and antibacterial activities. Therefore, selected actinomycetes showed higher antifungal activity against *C. albicans* than antibacterial activity. Our findings are consistent with several studies (37, 38, 39). They noticed that actinomycetes typically exhibit strong activity against Gram-positive bacteria but little action against Gram-negative bacteria. The actinomycete's outer membranes' morphological variations may cause varying sensitivity (4). Additionally, it is possible that Gram-negative bacteria in the earlier environment picked up the resistance genes from nearby resistant bacterial cells (40). Among the actinomycetes tested, strain U211 demonstrated the most potent antibacterial and antifungal activities against *E. coli* and *C. albicans*. The inhibition zone measured an average of 22.5 mm in the antibacterial assay and 23.3 mm in the antifungal assay. The ability of test isolates to produce the clear zone differed, presumably due to secondary metabolites produced (41) that can inhibit or kill other microorganisms. This could be potentially useful in the development of antibiotics or other therapeutic agents. However, further testing would be needed to confirm the antimicrobial activity of the compounds and evaluate their potential applications. It's also worth noting that large inhibition zones alone do not necessarily indicate the potency or effectiveness of the antimicrobial compounds produced by the actinomycete strain (42). The variation in clear zone diameter occurs because each isolate produces

different types of secondary metabolites having various chemical structures, compounds, and chemical concentrations (43).

CONCLUSION

Our findings demonstrate that the soil samples collected from flooded areas exhibit a diverse range of actinomycetes, as evidenced by their distinct morphological features. This observation implies that flooded soil areas may harbor a greater variety of actinomycetes than unflooded soil areas. Additionally, our study revealed that 57% of the tested actinomycete strains demonstrated activity against at least one test organism, highlighting their potential for future research.

ACKNOWLEDGMENT

The authors thank Ministry of Higher Education (MOHE) for the Fundamental Research Grant Scheme (FRGS) R/FRGS/A07.00/01121N00312016/000367) and Universiti Malaysia Kelantan, Jeli Campus for the laboratory facilities.

REFERENCES

1. Prudence SMM, Addington E, Castaco-Espriu L, Mark DR, Pintor-Escobar L, Russell AH, McLean TC. Advances in actinomycete research: an ActinoBase review of 2019. Microbiology (Reading, England). 2020;166(8): 683–694.
2. Oskay aM, Ьsame T, Cem A. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. African Journal of Biotechnology. 2005;3(9): 441–446.
3. Pandey B, Ghimire P, Agrawal VP. 2004. International Conference on the Great Himalayas: Climate, Health, Ecology, Management and Conservation, Kathmandu, Organized by Kathmandu University and the Aquatic Ecosystem Health and Management Society, Canada.
4. Pandey A, Ali I, Butola KS, Chatterji T, Singh V. Isolation and characterization of actinomycetes from soil and evaluation of antibacterial activities of actinomycetes against pathogens. International Journal of Applied Biology and Pharmaceutical Technology. 2011;2(4):384–392.
5. Nanjwade BK, Chandrashekhara S, Shamarez AM, Goudanavar PS, Manvi FV. Isolation and morphological characterization of antibiotic producing actinomycetes. Tropical Journal of Pharmaceutical Research. 2010;9(3):231–236.
6. Rahman MA, Islam MZ, Islam MAU. Antibacterial activities of actinomycete isolates collected from soils of Rajshahi, Bangladesh. Biotechnology Research International. 2011;857925.
7. Berdy J. Are actinomycetes exhausted as a source of secondary metabolites. Proceedings of the 9th Symposium Actinomycetes. 1995;13–34.
8. Agadagba SK. Isolation of actinomycetes from soil. 2014;4(3):136–140.
9. Jeffrey LS. Isolation, characterization and identification of actinomycetes from agriculture soils at Semongok, Sarawak. African Journal of Biotechnology. 2008;7: 3697-3702.
10. Selim MSM, Abdelhamid SA, Mohamed SS. Secondary metabolites and biodiversity of actinomycetes. Journal of Genetic Engineering & Biotechnology, 2021;19(1): 72.
11. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. Lancet, 2022; 399: 629–55.
12. Ventola CL. The antibiotic resistance crisis: Part 1: Causes and threats. Pharmacy and Therapeutics. 2015;40(4): 277.
13. Bush K, Courvalin P, Dantas G, Davies J, Eisenstein B, Huovinen P, Jacoby GA, Kishony R, Kreiswirth BN, Kutter E, Lerner SA, Levy S, Lewis K, Lomovskaya O, Miller JH, Mobashery S, Piddock LJ, Projan S, Thomas CM, Tomasz A, Tulkens PM, Walsh TR, Watson JD, Witkowski J, Witte W, Wright G, Yeh P, Zgurskaya HI. Tackling antibiotic resistance. Nature Review Microbiology. 2011; Nov 2;9(12):894-6.
14. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clinical Infectious Diseases. 2009 Jan 1;48(1):1-12. doi: 10.1086/595011. PMID: 19035777.
15. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD. Clinical practice guideline for the management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clinical Infectious Diseases. 2016 Feb 15;62(4):e1-50.
16. Naglik JR, Gaffen SL, Hube B. Candidalysin: Discovery and function in *Candida albicans* infections. Current Opinion in Microbiology. 2019; 52: 100–109.
17. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016 Apr; 6(2):71-79.
18. Gow NAR, Johnson C, Berman J, Coste AT, Cuomo CA, Perlin DS, Bicanic T, Harrison TS, Wiederhold N, Bromley M, Chiller T, Edgra K. The importance of antimicrobial resistance in medical mycology. Nature Communication. 2022; 13: 5352.
19. Ramage G, Martнnez JP, Lypez-Ribot JL. Candida biofilms on implanted biomaterials: A clinically significant problem. FEMS Yeast Research. 2006 Nov;6(7):979-86.
20. Lobanovska M, Pilla G. Penicillin's discovery and antibiotic resistance: Lessons for the future? The Yale Journal of Biology and Medicine. 2017 Mar

29;90(1):135-145.
21. Friedman CR, Hoekstra RM, Samuel M, Marcus R, Bender J, Shiferaw B, Reddy S, Ahuja SD, Helfrick DL, Hardnett F, Carter M, Anderson B, Tauxe RV; Emerging Infections Program FoodNet Working Group. Risk factors for sporadic Campylobacter infection in the United States: A case-control study in FoodNet sites. Clinical Infectious Diseases. 2004 Apr 15;38 Suppl 3:S285-96.
22. Clinical and Laboratory Standards Institute (CLSI). 2020. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. Clinical and Laboratory Standards Institute.
23. Muramatsu H, Shahab N, Tsurumi Y, Hino M. A comparative study of Malaysian and Japanese actinomycetes using a simple identification method based on partial 16S rDNA sequence. Actinomycetologica. 2003;17(2):33–43.
24. Zhang J, Zhang L. Improvement of an isolation medium for actinomycetes. Modern Applied Science. 2011;(2):124–127.
25. Yeng CM, Pahirulzaman KAK. Potential application of actinomycetes as natural fungicide. IOP Conf. Ser.: Earth Environ. Sci. 2021; 756: 012066.
26. Yin LA, Pahirulzaman KAK. 2020. Curry leaf as potential antimicrobial agent. In Curry Leaf (Murraya koenigii): The Story of Potential Miracle Plant. Lambert Academic Publishing. ISBN 978-620-0-50574-3.
27. Unger IM, Kennedy AC, Muzika RM. Flooding effects on soil microbial communities. Applied Soil Ecology. 2009;42(1):1–8.
28. Akpoveta VO, Osakwe SA, Ize-iyamu OK, Medjor WO, Egharevba F. Post flooding effect on soil quality in Nigeria: The Asaba, Onitsha experience. Open Journal of Soil Science. 2014;4:72–80.
29. Elhottova D, Kristufek V, Triska J, Chrastny V, Uhlirova E, Kalcik J, Picek T. Immediate impact of the flood (Bohemia, August 2002) on selected soil characteristics. Water Air Soil Pollution. 2002;173:177–193.
30. Forar LR, Ali E, Mahmoud S, Bengraa C, Hacine H. Screening, isolation and characterization of a novel antimicrobial producing actinomycete, Strain RAF 10. Biotechnology. 2007;6(4):489–496.
31. Fenical W, Jensen PR. Developing a new resource for drug discovery: Marine actinomycete bacteria. Nature Chemical Biology. 2006;2:666–673.
32. Kalakoutskii LV, Agre NS. Comparative aspects of development and differentiation in actinomycetes. Bacteriological Reviews. 1976;40:469–524.
33. Salas JA, Guijarro JA, Hardisson C. High calcium content in *Streptomyces* spores and its release as an early event during spore germination. Journal of Bacteriology. 1983;155: 1316–1323.
34. Fang BZ, Salam N, Han MX, Jiao JY, Cheng J, Wei DQ, Xiao M, Li WJ. Insights on the Effects of heat pretreatment, pH, and calcium salts on isolation of rare actinobacteria from Karstic Caves. Frontiers in Microbiology. 2017;8:1535.
35. Jiang Y, Cao YR, Zhao LX, Wang Q, Jin RX, He WX, Xue QH. Treatment of ultrasonic to soil sample for increase of the kind of rare actinomycetes. Acta microbiologica sinica. 2010;50:1094–1097.
36. Subramani R, Sipkema D. Marine rare actinomycetes: A promising source of structurally diverse and unique novel natural products. Marine Drugs. 2019;17(5):249.
37. Cwala Z, Igbinosa EO, Okoh AI. Assessment of antibiotics production potentials in four actinomycetes isolated from aquatic environments of the Eastern Cape Province of South Africa. African Journal of Pharmacy and Pharmacology. 2010;5:118–24.
38. Nurkanto A, Julisiono H, Agusta A, Sjamsuridzal W. Screening antimicrobial activity of actinomycetes isolated from Raja Ampat, West Papua, Indonesia. Makara Journal of Science. 2012;16:21–6.
39. Charousovб I, Medo J, Halenбrovб E, Javorekovб S. Antimicrobial and enzymatic activity of actinomycetes isolated from soils of coastal islands. Journal of Advanced Pharmaceutical Technology & Research. 2017;8(2):46–51.
40. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clinical Infectious Diseases. 2009;49:1749–55.
41. Ouchari L, Boukeskasse A, Bouizgarne B, Ouhdouch Y. Antimicrobial potential of actinomycetes isolated from the unexplored hot Merzouga desert and their taxonomic diversity. Biology open. 2019;8(2):bio035410.
42. Charousovб I, Medo J, Hleba L, Cнsarovб M, Javorekovб S. Antimicrobial activity of actinomycetes and characterization of actinomycin-producing strain KRG-1 isolated from Karoo, South Africa. Brazilian Journal of Pharmaceutical Sciences. 2019; 55:e17249.
43. Dharmawan IWE, Retno K, Made SP. Isolation of *Streptomyces* spp. In Bali Barat National Park and inhibition test to five diarrheagenic *Escherichia coli* strain. Biodiversitas Journal of Biological. 2009;13:1-6.