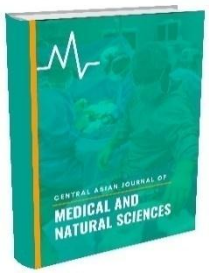
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 05 Issue: 01 | Jan-Feb 2024 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

PURIFICATION OF BIOACTIVE COMPOUNDS FROM *STREPTOMYCES* ISOLATES AND THEIR ANTIMICROBIAL ACTIVITY AGAINST SOME BACTERIA ISOLATED FROM FISH

1. Mohammed Mahdi Yaseen

Received 20th Nov 2023,
Accepted 28th Dec 2023,
Online 31st Jan 2024

[1] Department of Public Health, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq

Key words: Antibiotic resistance, community-acquired infections, nosocomial infections.

Abstract: Antibiotic resistance (AR) is one of the major health problems that challenges successful treatment of various types and degrees of infection, both in humans and animals. According to that, it is critically important to discover natural new compounds to control AR. The presented herein-study was conducted to extract bioactive compounds from some isolates of Streptomyces and to evaluate their antibacterial activity on some bacterial microorganisms isolated from fish. The work started with the use of river sediments as a source for the isolation of Streptomyces bacteria, which were identified further with the recruitment of PCR that targeted the 16S rRNA gene. Then, samples of fish kidney, intestines, gills, and skin were utilized at 1 gm each to isolate some bacteria. Bioactive compounds were alcoholic-based-extracted from Streptomyces isolates and further investigated using GC-MS assay. These bioactive chemicals were evaluated as antibacterial agents against the fish bacterial isolates by employing Mueller Hinton agar (MHA). The results revealed two isolates of Streptomyces sp. mqw1 (SM1) and Streptomyces sp mqw2 (SM2). The fish isolates were Vibrio sp., Aeromonas sp., E. coli, Enterobacter sp., Pseudomonas sp., Staphylococcus sp., Klebsiella sp., Salmonella sp., and Serratia sp. The findings of MHA demonstrated higher antibacterial activities from SM2 isolates when compared with those from SM1. The findings of the current study indicate important antibacterial activities of the bioactive compounds, which were isolated from Streptomyces, especially mqw2, against fish bacterial isolates.

Introduction

The worldwide health problem caused by the uncontrolled expansion of AR is mostly dependent on the excessive consumption of antibiotics. Nevertheless, there are additional reasons that are principally accountable for the rise in the incidence. The socioeconomics that contributes to these problems include inadequate community cleanliness, food safety issues, insufficient infection management measures in healthcare facilities, the presence of antibiotics in the environment, and their utilization in the animal and food sectors. The phenomenon of AR was initially recognized over 50 years ago (1). By the late 1950s, a majority of *S. aureus* cultures had acquired resistance to penicillin, which was previously the standard treatment for these infections. However, AR was not considered a significant global issue for an extended period. This was due to the development of new drug categories, including vancomycin and methicillin, in the 1960s (2).

These advancements led to the belief that the issue of AR could be resolved by creating new molecules. Regrettably, throughout the subsequent decades, microorganisms have acquired several distinct mechanisms of AR, which shield them from the impact of these medications. As a result, AR has progressed. The WHO initially released a catalogue of 12 bacterial families in 2017, which provide the most significant risk to human well-being. The WHO classifies pathogenic microbes into three priority groups: critical, high, and medium priority. This classification is based on the level of urgency in developing new medicines to effectively battle these diseases (3). The microorganisms classified as the most crucial species are bacterial infections that are resistant to many antibiotics. These germs represent significant risks to patients in health care facilities, nursing homes, and those who rely on healthcare equipment like ventilators and blood tubes. The critical-priority pathogens include *Acinetobacter, Pseudomonas, K. pneumoniae, E. coli, and Enterobacter* spp (4,5). These organisms exhibit resistance to many medicines and have the potential for causing serious and frequently deadly infectious illnesses, including septicemia and pneumonia. The high priority group comprises antibiotic-resistant microorganisms, such as *E. faecium* and *S. aureus*, that are resistant to many substances, including vancomycin and fluoroquinolones. The medium priority group comprises microorganisms that consist of *Streptococcus pneumoniae* and *Shigella*, which exhibit some level of resistance but may still be effectively eradicated by existing medicines (6).

Streptomyces, as a bacterial genus, is very significant due to its role as major producers of several antibiotics worldwide. They inhabit a wide range of settings, spanning from the depths of the sea to the peaks of high mountains. These bacteria belong to the phylum Actinobacteria and have the characteristics of being Gram-positive, filamentous, and capable of generating spores. Streptomyces separated from their nearest cousin, Kitasatospora, some 382 million years ago during the late Devonian era, which coincided with the emergence of terrestrial animals (7,8). Streptomyces are immobile bacteria that propagate by generating filamentous hyphae that infiltrate surfaces in pursuit of nourishment. Under situations of low resources, Streptomyces organisms generate aerial hyphae that undergo division, resulting in the production of spores. These spores possess the ability to withstand unfavorable conditions and may be readily transferred to new locations or sources of nutrition (9).

Throughout the growth stage, Streptomyces organisms synthesize secondary metabolites, which are chemicals that are not essential for their development or reproduction but can provide them with a competitive benefit. These compounds assist vegetative bacterial cells by preserving metals like iron (siderophores), shielding them from ultraviolet (UV) rays (by pigmentation), suppressing competition (antimicrobial agents), and promoting communication with other kinds of bacteria. Streptomyces is able to achieve molecular diversity due to its relatively big genome, that can be four times larger than the those of various bacteria (10).

AR is one of the major health problems that challenges successful treatment of various types and degrees of infection, both in humans and animals. According to that, it is critically important to

discover natural new compounds to control AR. The presented herein-study was conducted to extract bioactive compounds from some isolates of Streptomyces and to evaluate their antibacterial activity on some bacterial microorganisms isolated from fish.

Materials and methods

Isolation of *Streptomyces* bacteria from sediment and water sources.

Each of the samples underwent different handling processes. Initially, the samples underwent the removal of gravel and debris. After being air-dried, the samples were thoroughly combined and filtered through a 2-mm sieve. Dilutions were prepared utilizing sterile distilled water, with a maximum dilution factor of 10-5. To prepare the water specimens, 1ml of the sample was combined with 9ml of saline solution and mixed thoroughly. Preparations were made using dilutions ranging from 10-2 to 10-5.

one milliliter of each sample that was diluted was evenly distributed over selective media, including glycerol yeast extract agar, actinomycetes isolation agar, and trypticase soy agar supplemented with 6% NaCl. In addition, nalidixic acid was utilized at a concentration of 75µg/ml and cycloheximide at a concentration of 80µg/ml to suppress the growth of unwanted fungi and bacteria. The sample culture was conducted in three replicates and incubated for a period of 1-2 weeks at a temperature of 28±2°C. In order to achieve high levels of purity, the process of sub-cultivating the powdery and colored colonies was carried out using actinomycete isolation agar. The purified colonies obtained were stored in a solution of 10% glycerol at a temperature of -80°C. These colonies were regularly moved onto freshly made media for the purpose of investigation Baskaran et al (11).

The organism's development features and biochemical evaluations, including salt tolerance tests ranging from 1% to 12%, pH tolerance assays ranging from 2 to 12, and temperature tolerance assays at 10, 15, 20, 25, 30, 40, and 45ºC, were conducted following the procedures described by Boone et al (12). These experiments were conducted on SYE medium at 30ºC for a duration of 2-3 weeks.

Bacterial isolation from fish

A gram of the fish sample, consisting of the kidney, intestines, gills, and skin, was dissected and mixed thoroughly in a mortar. The substance was moved under sterile conditions to a sample container including nine milliliters of 0.1% sterile peptone water. The container was tightly sealed and vigorously shaken for a duration of 10 mins. It was then left undisturbed for a period of 20 mins. Following this, a 10-fold dilution process was carried out in duplicate from the separated supernatant. Afterward, a volume of 100 µL of the diluted mixture was placed onto separate agar medium. The number of viable aerobic bacterial colonies was then assessed using the standard plate count method, after incubating at 37ºC for 48 hours, according to the reported by Slabyj et al. (13). The quantification of coliform organisms and Gram-negative enteric bacteria was conducted utilizing pour plate methods, employing MacConkey agar and EMB Agar, respectively. The pathogenic Vibrio spp. were cultured on Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar. The plates were placed in an incubator set at a temperature of 35 ºC for a duration of 24 hours. The colony density was quantified using a Coulter Colony counter, employing the plate count approach. The mRS agar was utilized to isolate pathogenic Aeromonas spp., while Pseudomonas agar was applied for the purification of Pseudomonas spp. The microorganisms were identified by utilizing their morphological and biochemical properties, as earlier documented by Slabyj et al., (13).

16S rRNA gene amplification of bacterial isolates of Streptomyces

The *16S rRNA* gene was specifically identified utilizing the primers F: GT AGA T TGA TCC TGG CTC AG and R: GGT TAC CTT GTT ACG ACT T, as described by Lane et al (14). The DNA extracted was 10%-added to a 50µl-PCR reaction that comprised of each primer at a concentration of

0.4µM, each dNTP at a concentration of 0.2Mm, BSA at a concentration of 0.2µl, and DNA polymerase (Promega) at a concentration of 1U. The PCR thermal cycle settings consisted of a 5-minute denaturation step at 94°C, followed by 30 cycles of a 30-second major denaturation at 95°C, a 30-second annealing at 60°C, and a 2-minute major extension at 72°C. The reaction was concluded with a terminal extension step of 10 minutes at 72°C. A 1% agarose gel was prepared for electrophoresis, which was prepped with ethidium bromide.

Extraction of bioactive compounds

The research successfully isolated secondary bioactive chemicals generated by Streptomyces sp. during the ideal growth conditions. The active metabolites were obtained employing a modified version of the extraction techniques described by Jensen et al. (15). The broth culture produced (with an optical density of 4.5 at a wavelength of 600 nm) was subjected to centrifugation at a speed of 10,000 rpm for a duration of 15 minutes. The supernatant, devoid of cells, was collected and combined with an equal amount of ethyl acetate. The mixture was then placed on a rotary shaker and left overnight at a speed of 200 rpm. The initial extracts were obtained by collecting the solvent layer and subsequently evaporating it using a rotary evaporator. The addition of 100% DMSO was necessary to prepare a stock solution of Streptomyces sp. SDJ10 crude extract at a concentration of 50 mg/mL (w/v). Subsequently, this stock was subjected to testing in order to validate its capacity for identifying antibacterial activity.

Assessment of antibacterial activity

The well diffusion method was utilized for evaluating the antimicrobial capacity of the broth culture, in which wells with a diameter of 6.4 mm were made on MHA. The growth was thereafter added to each well until it reached maximum capacity. The plates were placed in an incubator and kept at a temperature of 37 ° C for a duration of 24 hours. The bioactivity was assessed by determining the width of inhibition zones (in millimeters).

Thin layer chromatography

The extract was subjected to thin layer chromatography (TLC) utilizing a TLC plate coated with silica gel 60 F254 (Merck). Experiments were conducted using nine distinct solvent systems to identify the optimal component (Table 1). The findings indicated that the most efficient separation was attained by the utilization of flash column chromatography (FCC). The chemicals were seen using UV-light.

Table 1: Solvent components for flash column chromatography

Solvent component	Ratio
n- Hexane: Chloroform (HC)	1:09
Chloroform: Acetone (CA)	1:01
Ethyl acetate: Methanol (EM)	7:03
Ethyl acetate: n-Hexane (EH)	6:04
Ethyl acetate: Chloroform (EC)	2:01
Ethyl acetate	1:00
n-Hexane	1:00
Ether	1:00
Chloroform	1:00

Identification of functional groups by employing Fourier transform infrared (FTIR)

The determination of functional moieties of two active substances from Streptomyces sp. was conducted utilizing FTIR, as reported by Augustine et al. (16). In summary, 1 mg specimen was

subjected to milling with 80 mg of KBr to produce a highly refined powder. The powder obtained was further compacted into a slim pellet for examining instrument (Perkin Elmer Spectrum BX). The spectra were scanned within the range of 400 to 4000 cm-1 and then displayed as intensity against wave number.

Gas chromatography-mass spectrometry (GC-MS)

The metabolically active portion of Streptomyces sp. was examined utilizing the GC SHIMADZU QP2010 system and a gas chromatograph that was connected to a mass spectrometer. The mass spectrometer was further supplied with an Elite-1 fused silica capillary column. The column was 0.25 mm, 30.0m, and 0.25 µm of diameter, length, and a film thickness, respectively. The column was comprised entirely of dimethyl polysiloxane. A GC-MS detection method was employed using an ionization energy of 70eV. The carrier gas employed was helium gas with a purity of 99.99%.

The flow rate remained consistent at 1.51 mL/min. The used volume for injection was 1µL. The ion generator was heated to 200 °C, while the injector was at 240 °C. The oven was originally set at 70 °C and maintained at this level for 2 minutes. Subsequently, there was an increase of 300 °C, which lasted for 10 minutes. Mass spectra were obtained at an energy level of 70 electron volts (eV), with a scan interval of 0.5s and a scan range of 40 to 1000m/z. The overall duration of the process was 35 minutes. The average peak area for each constituent was contrasted to the overall region to determine the proportional percentage quantity of each ingredient. The WILEY8, National Institute Standard and Technique (NIST08s), and FAME databases were utilized to analyze the GC-MS and determine the substance identity. The spectrum of the unidentified constituent was contrasted to the spectra of the previously identified constituent found in the WILEY8, NIST08s, and FAME library. An assessment was conducted on the test substance to determine its name, chemical formula, molecular weight, and constituent structure.

Results

The results revealed two isolates of *Streptomyces* sp. mqw1 (SM1) and *Streptomyces* sp mqw2 (SM2). These isolates can be shown in figure 1. In this figure, the PCR demonstrated amplification of *Streptomyces* sp. mqw1 (SM1) and *Streptomyces* sp mqw2 (SM2) at 1500bp.

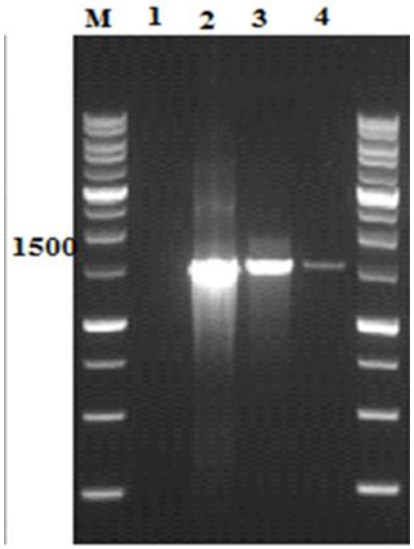


Figure 1: Agarose gel electrophoresis of *16S rRNA* gene-based PCR of *Streptomyces* isolated from sediment and water. Lane 1: 1kb ladder; lane 2: No-DNA control; lane 3: positive *E. coli* DNA-based control; lane 4: *Streptomyces* sp. mqw1; lane 5: *Streptomyces* mqw2 sp.

The fish isolates were *Vibrio* sp., *Aeromonas* sp., *E. coli*, *Enterobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Klebsiella* sp., *Salmonella* sp., and *Serratia* sp. The isolate percentages were 28%, 20%, 12%, 10%, 10%, 7%, 5%, 5%, and 3%, respectively. Figure 2 displays these bacterial species and their incidence rate in the organs of fish examined.

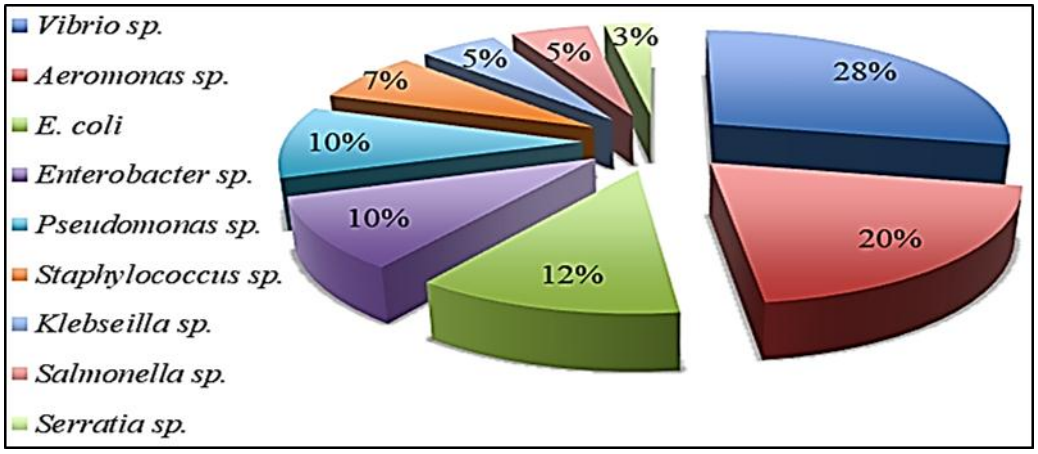


Figure 2: Bacterial species isolated from fish organs.

The TLC revealed the best systems of solvents that were utilized. The TLC highlighted the SM1 profile using different systems of solvents. The TLC findings are shown in Figure 3.

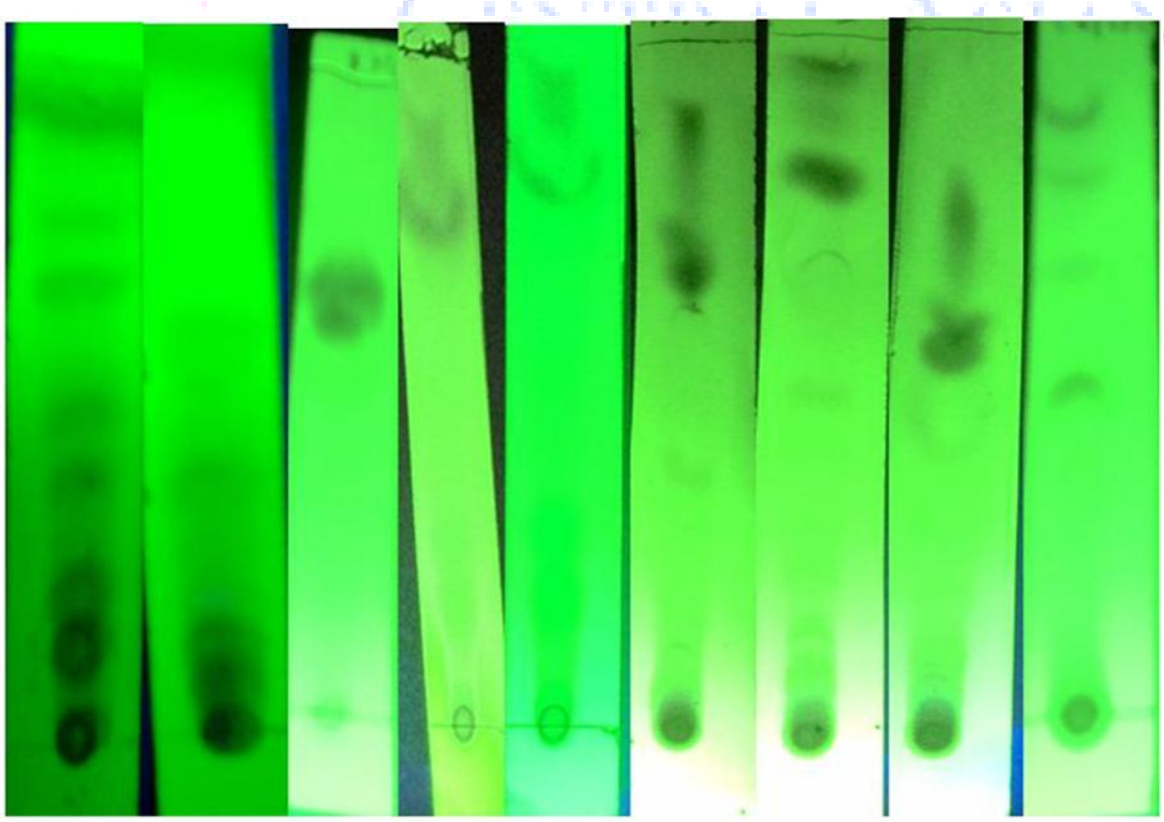


Figure 3: Profile of *Streptomyces* sp. mqw1 under different systems of solvents according to thin layer chromatography.

The FT-IR findings showed different bioactive groups. These groups were displayed as some analyzed peaks against the background. Figure 4 shows these reads of active substances.

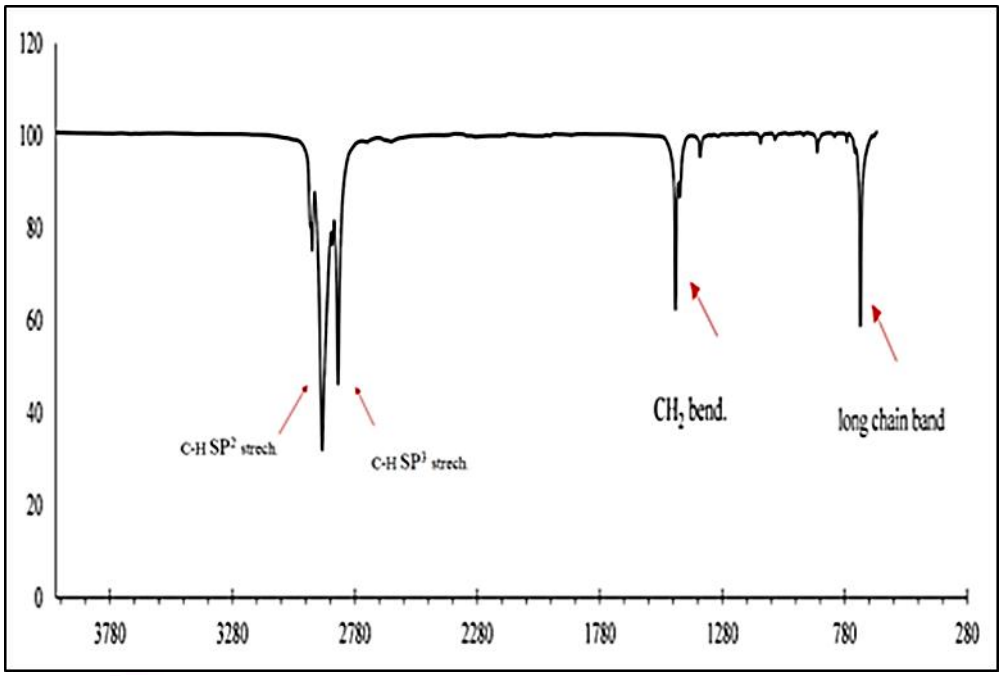


Figure 4: Active groups of *Streptomyces* sp. Isolated from sediments and water as revealed by the FTIR analysis.

The findings of MHA demonstrated higher antibacterial activities from SM2 isolates when compared with those from SM1. These zones of inhibition were high in

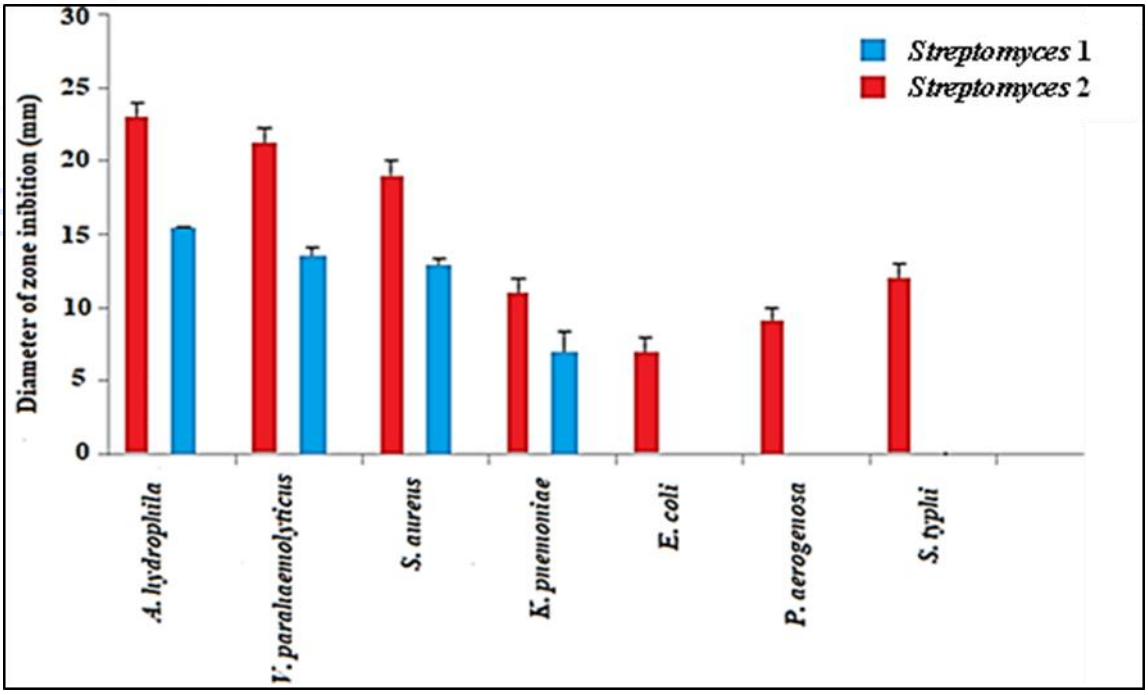


Figure 5: Antagonistic bioactivity of *Streptomyces* isolates against some bacterial isolates of fish.

Discussion

The current study findings revealed the extraction of some bioactive compounds from *Streptomyces* isolated from sediments and water. Several studies showed that this microorganism is an important source for active substances that introduce antimicrobial activity against a wide range of pathogenic

microbes. Previously to the 1970s, isolating novel substances from *Streptomyces* was extremely simple. However, since 1985, just three novel families of antimicrobial agents such as Platensimycin, derived from Streptomyces platensis. An isolate of thermotolerant *Streptomyces* sp. TM32, which produces antibiotics, was lately discovered. This is a newly discovered variant of *Streptomyces sioyaensis* that has potent antibacterial properties against several disease-causing microorganisms affecting humans and plants. It is particularly effective against *Staphylococcus haemolyticus* MR-CoNS (17,18).

Streptomyces often produce antimicrobial compounds through the utilization of complicated enzymatic systems such as polyketide synthases (PKSs), non-ribosomal peptide synthases (NRPSs), or a mix of these. These expansive multienzyme complexes employ several distinct motifs to carry out chemical alterations that can generate a diverse array of antimicrobial agents (19). Within the PKS system, antimicrobial agents often originate as a ketide monomer that is linked to an acyl carrier protein. The synthesis of the antibiotic occurs by a sequence of enzymatic reactions, usually incorporating acyltransferases, ketidesynthases, and other enzymes. These reactions result in the creation of the core structure of the polyketide antimicrobial agents. The antibiotic synthesis can undergo other alterations, such as cyclization, decarboxylation, dehydration, reduction, and methylation (20).

The findings of the current study align with the research conducted by Das et al. (21), which demonstrated that the ethyl acetate extract of the Streptomyces sp. strain EA-PWS52 contains biologically active substances including benzene acetic acid, pyrrolizidine derivatives, heterocyclic compounds, and other compounds that possess antimicrobial and antioxidant properties.

Rammali et al. (22) discovered that the ethyl acetate extract of the microorganism included diethyl trisulfide, which demonstrated potent antibacterial and antifungal capabilities; indole analogues such as Indole-3-carboxylic acid. This compound has been shown to possess a range of biological actions including anticonvulsant, anticancer, antibacterial, antitubercular properties; and pyrrolizidine based derivatives, especially Pyrrolo [1,2-a]pyrazine-1,4-dione. These substances are natural heterocyclic chemicals that have shown antibacterial properties.

Desbois and Lawlor (23) documented the antibacterial effects of dihomo-γ-linolenic acid (DGLA) on gram-positive microorganisms, namely Propionibacterium acnes and S. aureus. The minimum inhibitory concentration readings for DGLA were found to be 128 mg/L and 1024 mg/L, respectively. There are reports of DGLA exhibiting antibacterial properties.

The findings from the molecular recognition, which relied on analyzing the similarity of *16S rRNA* gene, align with prior research and validate that *Streptomyces* is the prevailing species in sediments in comparison to other Actinomycetes genera. Kitouni et al. (21) found that 93% of Actinomycetes effective isolates are classified within the Streptomyces genus.

Conclusion

The findings of the current study indicate important antibacterial activities of the bioactive compounds, which were isolated from *Streptomyces*, especially mqw2, against fish bacterial isolates.

References

1. Coculescu BI. Antimicrobial resistance induced by genetic changes. J Med Life [Internet]. 2009 Apr 4 [cited 2024 Jan 26];2(2):114–23. Available from: /pmc/articles/PMC3018982/
2. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, et al. Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist [Internet]. 2018 [cited 2024 Jan 26];11(10):1645–58. Available from: /pmc/articles/PMC6188119/

3. Collignon P, Beggs JJ. Socioeconomic Enablers for Contagion: Factors Impelling the Antimicrobial Resistance Epidemic. Antibiotics [Internet]. 2019 Sep 1 [cited 2024 Jan 26];8(3):86. Available from: /pmc/articles/PMC6784211/

4. Tigabu A, Getaneh A. Staphylococcus aureus, ESKAPE Bacteria Challenging Current Health Care and Community Settings: a Literature Review. Clin Lab [Internet]. 2021 Jul 1 [cited 2024 Jan 26];67(7):1539–49. Available from: https://pubmed.ncbi.nlm.nih.gov/34258960/

5. Davin-Regli A, Pagès JM. Enterobacter aerogenes and Enterobacter cloacae; versatile bacterial pathogens confronting antibiotic treatment. Front Microbiol [Internet]. 2015 [cited 2024 Jan 26];6(5):3922. Available from: /pmc/articles/PMC4435039/

6. McGuinness WA, Malachowa N, DeLeo FR. Focus: Infectious Diseases: Vancomycin Resistance in Staphylococcus aureus. Yale J Biol Med [Internet]. 2017 Jun 1 [cited 2024 Jan 26];90(2):269–81. Available from: /pmc/articles/PMC5482303/

7. de Lima Procópio RE, da Silva IR, Martins MK, de Azevedo JL, de Araújo JM. Antibiotics produced by Streptomyces. Braz J Infect Dis [Internet]. 2012 Sep [cited 2024 Jan 26];16(5):466–71. Available from: https://pubmed.ncbi.nlm.nih.gov/22975171/

8. Chater KF. Streptomyces inside-out: a new perspective on the bacteria that provide us with antibiotics. Philosophical Transactions of the Royal Society B: Biological Sciences [Internet]. 2006 May 5 [cited 2024 Jan 26];361(1469):761–8. Available from: /pmc/articles/PMC1609407/

9. Chater KF. Recent advances in understanding Streptomyces. F1000Res [Internet]. 2016 [cited 2024 Jan 26];5:2795. Available from: /pmc/articles/PMC5133688/

10. Bibb MJ. Regulation of secondary metabolism in streptomycetes. Curr Opin Microbiol [Internet]. 2005 [cited 2024 Jan 26];8(2):208–15. Available from: https://pubmed.ncbi.nlm.nih.gov/15802254/

11. Baskaran R, Vijayakumar R, Mohan PM. Enrichment method for the isolation of bioactive actinomycetes from mangrove sediments of Andaman Islands, India. Malays J Microbiol. 2011;7(1):26–32.

12. Boone D, CR and GG. Bergey's Manual® of Systematic Bacteriology. New York, NY: Springer New York; 2001.

13. SLABYJ BM, MARTIN RE, RAMSDELL GE. Reproducibility of Microbiological Counts on Frozen Cod: A Collaborative Study. J Food Sci [Internet]. 1981 May 1 [cited 2024 Jan 26];46(3):716–9. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2621.1981.tb15333.x

14. Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc Natl Acad Sci U S A [Internet]. 1985 [cited 2024 Jan 26];82(20):6955–9. Available from: /pmc/articles/PMC391288/?report=abstract

15. Jensen JS, Blachez B, Egebo M, Meyer AS. Rapid Extraction of Polyphenols from Red Grapes. Am J Enol Vitic [Internet]. 2007 Dec 1 [cited 2024 Jan 26];58(4):451–61. Available from: https://www.ajevonline.org/content/58/4/451

16. Augustine SK, Bhavsar SP, Kapadnis BP. A non-polyene antifungal antibiotic from Streptomyces albidoflavus PU 23. J Biosci [Internet]. 2005 [cited 2024 Jan 26];30(2):201–11. Available from: https://pubmed.ncbi.nlm.nih.gov/15886456/

17. Martens E, Demain AL. Platensimycin and platencin: promising antibiotics for future application in human medicine. J Antibiot (Tokyo) [Internet]. 2011 Nov [cited 2024 Jan 26];64(11):705–10. Available from: https://pubmed.ncbi.nlm.nih.gov/21915133/

18. Nakaew N, Lumyong S, Sloan WT, Sungthong R. Bioactivities and genome insights of a thermotolerant antibiotics-producing Streptomyces sp. TM32 reveal its potentials for novel drug discovery. Microbiologyopen [Internet]. 2019 Nov 1 [cited 2024 Jan 26];8(11):e842. Available from: /pmc/articles/PMC6854843/

19. Felnagle EA, Jackson EE, Chan YA, Podevels AM, Berti AD, McMahon MD, et al. Nonribosomal Peptide Synthetases Involved in the Production of Medically Relevant Natural Products. Mol Pharm [Internet]. 2008 Mar [cited 2024 Jan 26];5(2):191–211. Available from: /pmc/articles/PMC3131160/

20. Risdian C, Mozef T, Wink J. Biosynthesis of Polyketides in Streptomyces. Microorganisms [Internet]. 2019 May 1 [cited 2024 Jan 26];7(5):124. Available from: /pmc/articles/PMC6560455/

21. Kitouni M, Boudemagh A, Oulmi L, Reghioua S, Boughachiche F, Zerizer H, et al. Isolation of actinomycetes producing bioactive substances from water, soil and tree bark samples of the north–east of Algeria. J Mycol Med. 2005 Mar 1;15(1):45–51.

22. Rammali S, Hilali L, Dari K, Bencharki B, Rahim A, Timinouni M, et al. Antimicrobial and antioxidant activities of Streptomyces species from soils of three different cold sites in the Fez-Meknes region Morocco. Sci Rep [Internet]. 2022 Dec 1 [cited 2024 Jan 26];12(1):17233. Available from: /pmc/articles/PMC9568536/

23. Desbois AP, Lawlor KC. Antibacterial Activity of Long-Chain Polyunsaturated Fatty Acids against Propionibacterium acnes and Staphylococcus aureus. Mar Drugs [Internet]. 2013 [cited 2024 Jan 26];11(11):4544–57. Available from: /pmc/articles/PMC3853744/