Journal of Basic and Environmental Sciences

ISSN
Online: 2356-6388
Print: 2536-9202

Research Paper Open Access

Bioactive secondary metabolites from marine actinomycetes and their inhibitory effect on bacteria of Drinking water

Mohamed E. Elawady [1], Mervat G. Hassan [2], Ahmed A. Hamed [3], Hatem E. Elbeih [2], Soheir S. Abdelsalam [2].

[1] Microbial Biotechnology Department, National Research Centre, El- Buhouth Street, Dokki, Cairo, Egypt.

[2] Microbiology Department, Faculty of Science, Benha University, Benha, Egypt.

[3] Microbial Chemistry Department, National Research Centre, El-Buhouth Street, Dokki, Cairo, Egypt.

➢ **Corresponding author E-mail address: mervat.hassan@fsc.bu.edu.eg**

Abstract

This study focuses on the isolation of marine actinomycetes, their cultivation through fermentation on rice medium, and the subsequent evaluation of their antimicrobial activity. Marine environments are known to harbor diverse and potentially valuable microorganisms, including actinomycetes, which are renowned for their capacity to produce bioactive compounds.

Marine actinomycetes were isolated from marine samples, and their cultivation was carried out using a rice-based medium. This fermentation process aimed to stimulate the production of biologically active secondary metabolites. These metabolites are of particular interest due to their potential antimicrobial properties, which could have significant applications in the field of medicine and biotechnology.

The isolated actinomycetes' rice medium extracts were carefully examined for their antimicrobial activity. The study assessed their effectiveness in inhibiting the growth of various microorganisms, highlighting their potential as a source of novel bioactive compounds with applications in the field of antimicrobial agents. The results of this investigation contribute to our understanding of the bioresources present in marine ecosystems and their potential for developing new therapeutic agents.

Keywords: Antimicrobial activity, Secondary metabolites, Actinomycetes, *Streptomyces*.

Introduction

Marine ecosystems have long captivated the attention of researchers due to their exceptional biodiversity and the potential for discovering novel bioactive compounds with diverse applications. Among the microorganisms inhabiting marine environments, actinomycetes have emerged as a prolific source of bioactive secondary metabolites, valued for their therapeutic and biotechnological significance.

Actinomycetes, a group of Gram-positive bacteria, have been prolific producers of bioactive secondary metabolites such as antibiotics, anticancer agents, and immunosuppressants. These compounds have revolutionized the pharmaceutical industry and significantly advanced medical science (Bérdy, 2012) **[1]**. Marine actinomycetes, in particular, have gained prominence due to their ability to thrive in extreme environments, which may influence the biosynthesis of unique secondary metabolites with potent biological activities (Bhimba, 2015) **[2]**.

Drinking water safety is of paramount importance, as contaminated water can pose severe health risks to the population. Bacterial contaminants, including those capable of forming biofilms, are a recurring concern in the maintenance of water quality and safety. Biofilms are structured communities of microorganisms that adhere to surfaces and are often implicated in the deterioration of water quality by harboring pathogenic bacteria and facilitating resistance to conventional disinfection methods (Flemming et al., 2016) **[3]**.

Recent research has explored the potential of marine actinomycetes and their secondary metabolites to address this issue by inhibiting the growth and biofilm formation of waterborne bacteria. These studies have demonstrated the antimicrobial properties of compounds produced by marine actinomycetes and their potential as innovative solutions to mitigate bacterial contamination in drinking water (Jog et al., 2019; Mandal et al., 2018) **[4,5]**.

This review seeks to consolidate the existing body of knowledge on bioactive secondary metabolites from marine actinomycetes and their inhibitory effects on bacteria found in drinking water. By examining the latest research findings in this field, we aim to shed light on the promising potential of marine actinomycetes-derived compounds in safeguarding the microbiological quality of drinking water sources and contributing to the development of novel water treatment strategies.

Material and methods

Water Sample Collection at Different Drinking Water Treatment Stages

Water samples were meticulously collected for bacteriological analysis from distinct points within the drinking water treatment plant, encompassing the following stages: raw water, sedimentation/clarification, filtration, and disinfection. This sampling took place at the Dakahlia Drinking Water Company, specifically at the New Mansoura water plant situated in Mit-Khamis, Dakahlia governorate, Egypt. Each of these stages yielded fourteen samples, all carefully preserved in 250-500 mL sterilized glass containers. Subsequently, these samples were stored in an icebox, maintained at a temperature range of 2-8°C, and kept for a holding period of 6-8 hours.

Isolation and Purification of Bacterial Isolates

The isolation process was conducted using the serial dilution method, utilizing buffered water. Specifically, a 1 mL sample was combined with 9 mL of sterile diluted water (buffered water) in a test tube. The serial dilution procedure was then executed until the optimal bacterial/cell dilution of 10^{-5} (1 in 100,000) was achieved. Isolation was further carried out through the membrane filter technique, involving the use of membrane filter paper. These filter papers were placed onto sterile Petri dishes (6 cm) containing R2A agar media with utmost sterility. Following preparation, the samples were incubated at 35°C ± 0.5 for duration of 24 hours.

Collection of Marine Samples

Marine samples, comprising both marine water from depths of ±3-5 meters and sediment water, were procured from varied locations along the Red Sea beach in Hurghada City. These samples were carefully preserved at 4°C pending further laboratory analysis, with aseptic transport to the lab conducted in dry, sterile, insulated containers.

Isolation and Purification of Streptomycetes

To isolate streptomycetes, 10 mL of sediment water and marine water samples were introduced into 90 mL of sterilized saline solution (0.85% w/v aqueous NaCl solution). The suspensions were then plated using the serial dilution technique. Petri dishes were prepared the day before plating, with overnight incubation at 37°C to remove moisture from the agar surface (Shearer, 1987) **[6]**. Subsequently, 0.1 mL of the appropriate diluted inoculum was evenly distributed on each Petri dish. Isolation of streptomycetes from marine samples incorporated the use of 50% seawater in the isolation medium.

Preparation of Streptomycetes Extracts

Streptomyces isolates (HA1-HA12) were cultivated on solid rice media in small volumes for duration of 7 days at 30°C, employing 100 g of commercial rice and 150 mL of 50% natural seawater. Following incubation, the culture media of these strains were individually subjected to ethyl acetate extraction, followed by decantation and filtration. The resultant organic extracts were then concentrated under vacuum conditions and subsequently employed for biological antimicrobial testing.

Antimicrobial Activity Screening

The assessment of antibacterial activity for the various extracts (HA1–HA12) was carried out using the disk diffusion method. This experiment was conducted on 96-well flat polystyrene plates. A total of 10 µL of test extracts (resulting in a final concentration of 500 µg/mL) were combined with 80 µL of lysogeny broth (LB broth) and 10 µL of a suspension of an isolated bacterial culture in the log phase. Subsequently, the plates were incubated at 37°C for an overnight duration. The effective antibacterial activity of the test substances was indicated by clear zones around the wells, while substances lacking such effects resulted in opaque growth media within the wells. Untreated pathogens were used as the control group, and the absorbance was measured at OD600 after approximately 20 hours using a SPECTROstar Nano-Microplate Reader from BMG LABTECH GmbH in Allmendgrun, Germany.

Results

Isolation of bacteria from water

A total of twenty-three bacterial isolates were successfully retrieved from various stages of the water treatment process **(Table 1)**. For the cultivation and purification of these bacterial isolates, they were cultivated on R2A agar media using the membrane filtration technique.

Subsequently, all of the samples, derived from the different stages of water treatment, were subjected to the purification process on R2A agar media. This comprehensive approach facilitated the successful retrieval and isolation of the bacterial isolates, each of which could potentially contribute valuable insights into the water treatment and purification process.

Table (1): Bacterial isolates from various stages of the water treatment process

Purification steps	Bacterial Isolates
Raw water step	14 Isolates
Clarification step	8 Isolates
Filtration step	1 Isolates
Disinfection step	0 Isolates

Isolation, fermentation and extraction of actinomycetes

Marine specimens were gathered from the shores of the Red Sea in Hurghada, Egypt. From this collection, a total of twelve streptomycetes were isolated. These isolated actinomycetes strains were then cultivated on a rice-based medium in a small-scale fermentation process, aimed at obtaining the bioactive constituents they may produce. Subsequently, the cultures were subjected to extraction using ethyl acetate. To prepare the ethyl acetate phase for subsequent analysis, the solvent was evaporated until it was completely devoid of any residual liquid.

The antimicrobial activity screening of different crude extracts

The antimicrobial activity of the isolated actinomycetes acetate extracts was evaluated. Several isolates exhibited antimicrobial activity against 23 bacterial isolates but one isolate (HA7) from the 12 streptomycetes isolates showed potent antimicrobial and was the most effective. While most crude isolates have variable activity against 23 bacterial isolates **(Figure 1)**.

Bacterial inhibition ratios, ranging from RB1 to FB1, show great variation.

For bacteria RB1, RB2, and RB3, they showed high inhibitory effects of 87%, 90%, and 87%, indicating strong antimicrobial activity of the antimicrobial agent (HA7). In contrast, bacteria RB4 to RB8 showed moderate inhibition, indicating reasonable inhibition of bacterial growth, as bacteria RB4 showed moderate inhibition of 85%, while bacteria R8 showed relatively weaker inhibition of 85%.

The values differed for bacteria RB9 to RB14, as RB11 showed a strong inhibition of 94%, while RB12 showed a relatively weaker inhibition of 67%.

The values also differed for bacteria CB1 to FB1, as CB7 bacteria showed a strong inhibition of 94%, while CB1 bacteria showed a relatively weaker inhibition of 53%, while FB1 bacteria showed a moderate effect of 69%.

This indicates that an antimicrobial agent (HA7) is characterized by a high total inhibition rate of up to 83%, which indicates its remarkable effectiveness in inhibiting the growth of various types of bacteria. These findings hold significance for potential antimicrobial agents or therapeutics and underscore the need for further investigation into the compounds or conditions responsible for these inhibitory effects, with potential applications in areas such as pharmaceuticals and drinking water treatment processes.

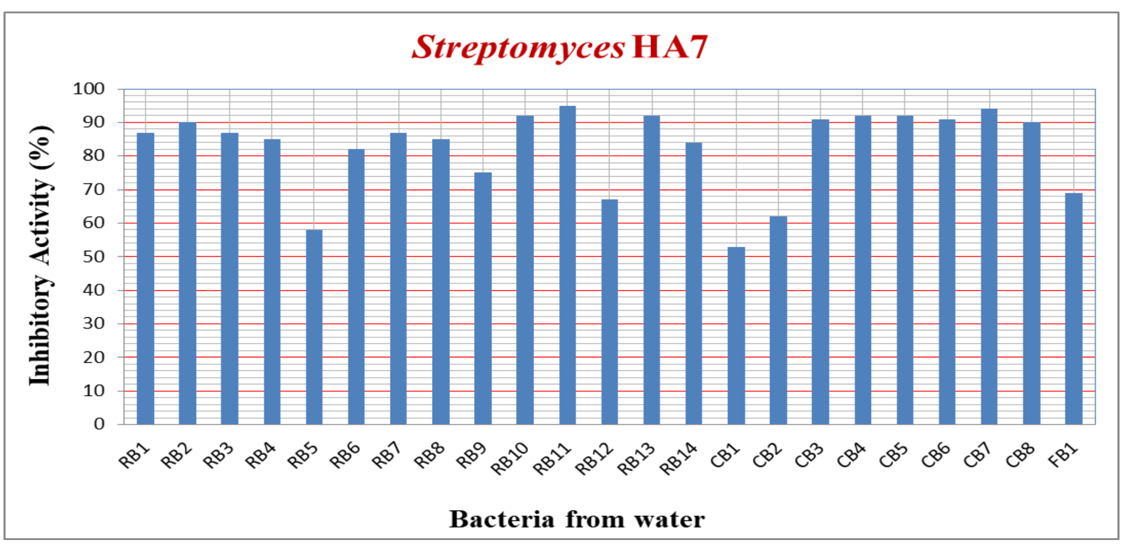

Figure (1): Antimicrobial activity of natural extracts from Streptomyces

Discussion

The biological treatment process was initially introduced in the early 20th century **[7]**, but drinking water was not purified using it.

Although biological drinking water treatment has been around since the 1800s **[8]**, the global scope of its utilization is still quite small.

There have only been a few studies conducted in nations that are developing as compared to some industrialized nations, such as the United States, which recently adopted biological drinking water treatment for the treatment of clean and safe drinking water.

Conventional treatment methods are unable to eliminate all pollutants, including those with high concentrations of microorganisms.

Natural products are organic chemicals that do not directly affect an organism's normal processes of growth, development, or reproduction according to Fraenkel et al. (1959) **[9]**. Everything in our lives depends on them. Natural resources have traditionally been important in the development of new active chemicals.

About 70 to 80 percent of all isolated chemicals during this time were antibiotics, the majority of which were derived from Streptomyces species and were mostly effective against bacteria and fungus. Discovery of anticancer, antiviral, and non-antibiotic enzyme inhibitory metabolites had only begun during this period (Berdy, 2005) **[10]**.

Actinomycetes have been found to have apparent antibacterial activity against practically all tested pathogenic strains, according to several investigations undertaken by experts.

In their previous studies, many actinomycetes isolates have been discovered by Abdelrazek (2014) **[11]**, who has also tested them for their capacity to create antibacterial chemicals. Marine *Streptomyces* sp., one of these strains, had strong antibacterial activity.

In our work, the bioactive components of *Streptomyces* sp strain HMA (HA7) were grown on rice medium, subjected to extensive fermentation, fractionated, and primarily divided into seven fractions by flash column chromatography from the extracted product.

By assessing their antibacterial activity against Enterobacter strain, the biological properties of each of the seven fractions were examined.

Based on the antibacterial results, the most active fraction (Fr1) was structurally determined using GC-Ms.

The produced pure compounds demonstrated antibiofilm action against *Enterobacter cloacae*, with an inhibition ratio of 69.3%, in addition to high percentages of antibiotic activity against other types of bacteria **(Figure 1)**.

These findings suggest that more investigation is necessary to create antimicrobial compounds or therapies that might be used in a variety of industries, including medicines and water purification.

Conclusion

In conclusion, actinomycetes produce potent antimicrobial compounds, which aid in the development of antibiotics and antimicrobial agents. Their bioactive metabolites are essential in combating bacterial infections and treating drug resistance.

Data on inhibition ratios ranging from RB1 to FB1 provide valuable insights into antimicrobial activity against bacteria.

The results indicate a wide range of inhibitory effects, ranging from high to moderate inhibition to low inhibition as well as varied inhibition ratios highlighting the diversity in antimicrobial efficacy against different bacterial isolates from drinking water.

This indicates that the antimicrobial agent HA7 showed significant inhibition against various bacterial isolates, confirming its potential as a powerful inhibitory agent against bacteria. These results indicate a promising avenue for developing antimicrobial agents or therapeutics with potential applications in various fields, including pharmaceuticals and drinking water treatment. Further research is warranted to identify the specific compounds or conditions responsible for these inhibitory effects and to explore their broader practical implications.

References

1. Bérdy, J. (2012). Antibiotics from actinomycetes: A comprehensive review. The Journal of Antibiotics, 65(11), 545-595.
2. Bhimba, A. (2015). Exploring marine actinomycetes: A treasure trove of bioactive secondary metabolites. Marine Drugs, 13(8), 5110-5123.
3. Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: An emergent form of bacterial life. Nature Reviews Microbiology, 14(9), 563-575.
4. Jog, S. P., Patil, S. S., & Datar, V. V. (2019). Marine actinomycetes: A source of potential antibacterial compounds for drinking water treatment. Environmental Science and Pollution Research, 26(2), 1331-1341.
5. Mandal, S., Kishi, L., Krieg, N. R., & Dyer, D. W. (2018). Marine actinomycetes-derived secondary metabolites and their antimicrobial properties. Marine Biotechnology, 20(5), 511-525.
6. Shearer, M. C. (1987). Methods for the isolation of non- streptomycetes actinomycetes. Journal of Industrial Microbiology. 2: 91– 97.
7. Carollo, Drinking Water Biological Treatment, (2014) (accessed 20 May 2015), http://www.carollo.com/sites/default/files/docs/BiologicalTreatment.pdf.
8. P.J. Evans, E.M. Opitz, P.A. Daniel, C.R. Schulz, Biological Drinking Water Treatment Perceptions and Actual Experiences in North America, Water Research Foundation and Department of Defense, Colorado, USA, 2010, p. 29.

9. Fraenkel, G. S. (1959). "The raison d'Etre of secondary plant substances". Science. 129 (3361): 1466–1470.

10. Bérdy, J. (2005). Bioactive microbial metabolites. The Journal of Antibiotics. 58: 1–26.

11. Abdel-Razek, A.S. (2014). Microbiological and chemical studies on some bioactive secondary metabolites from certain. Streptomycetes. PhD Thesis: Zagazig University.