International Journal of Research in Engineering, Science and Management
Volume 5, Issue 6, June 2022
https://www.ijresm.com | ISSN (Online): 2581-5792

Isolation of Antibiotics from Marine Microflora

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***Abstract*: Antibiotics are life-saving drugs that enhance our survival. Excessive use of these Antibiotics has caused drug resistance and it is important for us to discover new antibiotics. In my project, I have tried to prove that Marine microflora can be used as an Antibiotic. Samples were collected from the coast of the Bay of Bengal and by pour plate method after incubating it for 7 days at 25^0C, growth of some white colonies with a powdery appearance were observed which probably are Actinomycetes. Later, an Antibiotic sensitivity test was done to check if these colonies (Actinomycetes) inhibit the growth of *E.coli*. After 28 hours we were able to see a clear zone of inhibition. Therefore, Antibiotics can be made from Marine microflora and this might overcome the problem of drug resistance.**

***Keywords*: Antibiotics, Marine, Microflora.**

1. Introduction

The marine environment occupies 71 % of the earth's surface and the future of the world population depends on this environment for its food and other lifesaving wonder drugs. Hence, the utilization of marine resources for developmental purposes has gained considerable attention in recent times. The biological diversity of the marine environment offers enormous scope for the discovery of novel natural products. A huge variety of microflora can be isolated and taken as a species of interest but the promising results with Actinomycetes isolates can prove to be a potential source. Actinomycetes can be taken as a source of antibiotics

About 90 % of Antibiotics are produced by Actinomycetes.

Hypothesis:

- My hypothesis is to prove that marine microflora (probably actinomycetes), can be used as an antibiotic.
- To also prove that Actinomycetes is sensitive to bacteria.

Statement of problem:

Excessive use of Antibiotics creates drug resistance to the most commonly used antibiotics like Tetracycline.

Research question:

Can we discover new antibiotics from marine offshore sediments??

2. Methods

Independent variables:

- Marine sediment samples

Dependent variable:

- Growth of Microflora.

Controlled variable:

- Sterile seawater
- Nutrient agar
- Mueller Hilton Agar
- Timing
- Temperature

Materials:

- Marine sediment samples
- Sterile sea water
- Agar medium 1 – Nutrient agar
- Agar medium 2 – Mueller Hilton agar
- Petri plates
- Aqua clave
- Incubator
- Loop
- Cotton swab
- Antibiotic disc
- Scale

1) *Sample collection*

- Marine sediment samples are collected from the brackish soil deposits near the coast of the Bay of Bengal from three different areas in a sterile airtight container.
- Seawater is also collected.
  Bay of Bengal: Marina beach, Chennai.

- The samples were stored at 3 degree Celsius for a day until they were treated.

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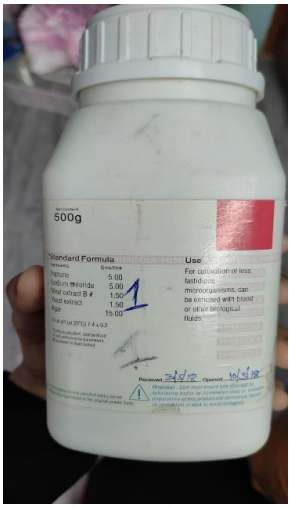
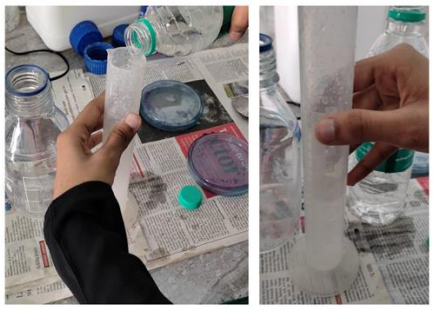
2) Sample preparation

The procedure of Pour plate technique:

- Preparation of Nutrient agar
- Adding 50 ml of water to 1.4 grams of agar powder.





Heat treatment:

- Heating of the soil samples in an autoclave at 80 degrees Celsius.

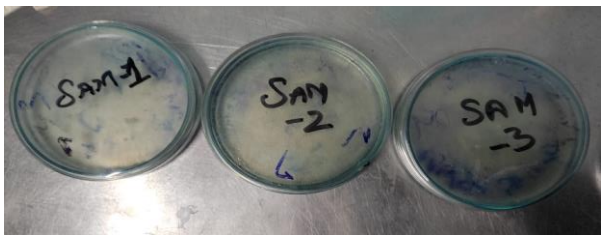
Sterilization of samples:

- Sterilization of the Nutrient Agar, seawater, and Petri plates in an autoclave at 120 degrees Celsius.

- Sterilization of the Nutrient Agar, seawater, and Petri plates in an autoclave at 120 degrees Celsius.

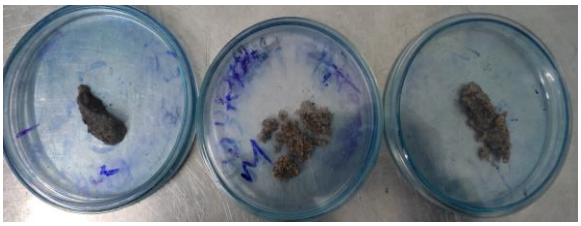
Making of the Agar plates:

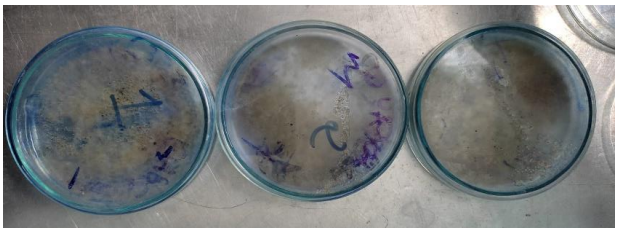
- Named the Petri plates as sample 1, sample 2, and sample 3.
- Then Nutrient agar solution was poured into the Petri plates and was left untouched until it was set.



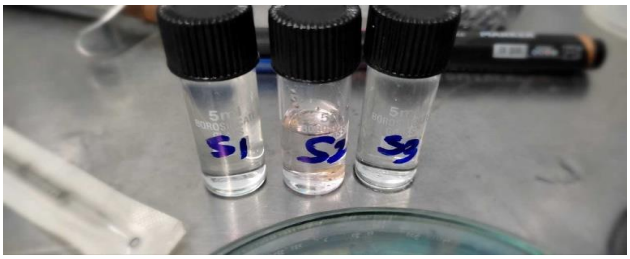
Diluting sediment samples with seawater:

- 1 Gram of sample was placed on the Petri plates and was diluted with 10ml of sterile seawater.





- The sediment sample was poured into mini bottles.



Pouring the samples onto the nutrient Agar:

- The sediment sample is poured primarily onto nutrient agar.

Incubating the samples:

The plates are incubated at 25 degrees Celsius for 10 days and observed on day 3, day 7, and Day 10.

Results of pour plate method:
Pour plate method:
Colony morphology:

- A tough or powdery texture colonies appear on the agar surface area (Might be Actinomycetes)

Pictures captured on day 7

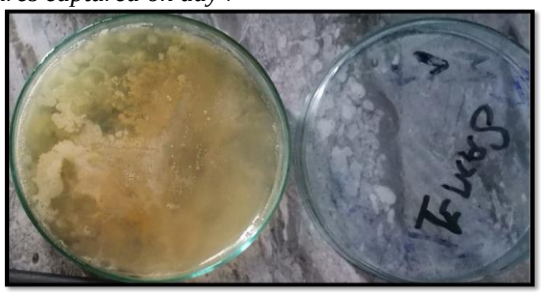
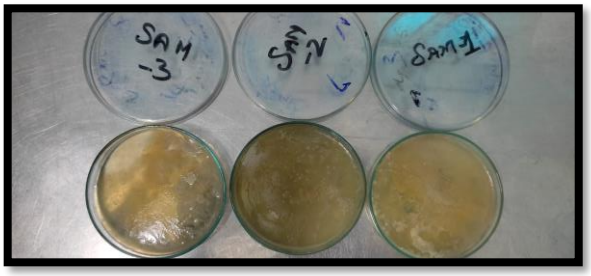


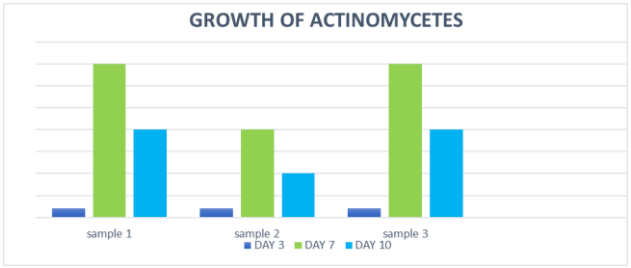
Table 1
Isolation of pure culture (Streaking methods)

No. of colonies	3 days	7 days	10 days
Nutrient agar sample 1	No growth	Yes, can see 7 – 10 round white colonies with a powdery appearance.	Fewer colonies when compared to DAY 7
Nutrient agar sample 2	No growth	Yes, can see 5– 7 round white colonies with a powdery appearance	Fewer colonies when compared to DAY 7
Nutrient agar sample 3	No growth	Yes, can see 7- 10 round white colonies with a powdery appearance	Fewer colonies when compared to DAY 7

DAY 10:



Comparative analysis of 3 samples:



DAY 3 – no growth.

The number of colonies in DAY 7 is more when compared to DAY 10.

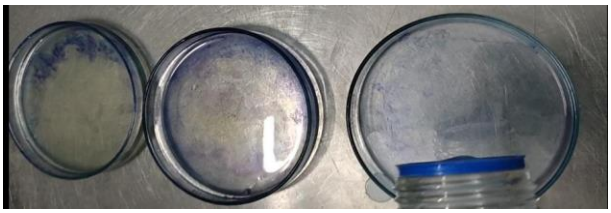
Antibiotic sensitive test:

1. Making of antibiotic disc:

- Heat the loop and pick a few colonies from the agar plate culture and suspend these in sterile saline

- Repeat the same for all three samples.
- When the suspension is at the correct turbidity, put the sterile disc in Saline solution.

2. Preparation of Mueller agar plates:



- Prepare Mueller Hinton Agar and sterile it.

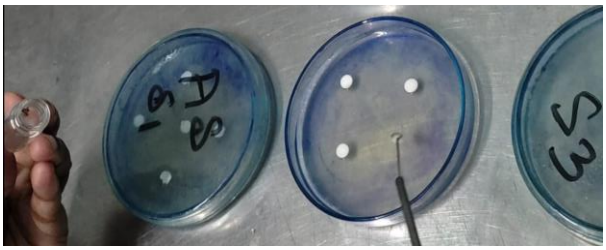
- After sterilization put the Mueller Hinton agar solution into 3 Petri plates and leave it until it sets.

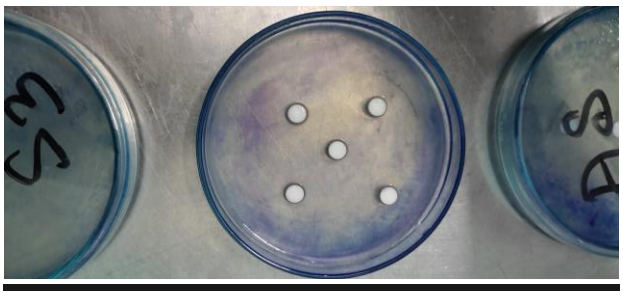
3. Swap method:

- Now take some microbial colonies (*E.Coli*) with the help of a cotton swab and Gently rub the end of the cotton swab on the agar.

4. Placing the antibiotic disc:

- Ensure that you press the disc down onto the agar so that it is secure and does not fall off.





- After applying the antibiotic discs, you should aim to get the plates into the incubator within 15 min.

5. Zone of inhibition

- Then zone of inhibition caused by the antibiotic is measured after 20 hours.
- It gives an idea of how effective the antibiotic is against a particular bacterium.

3. Results

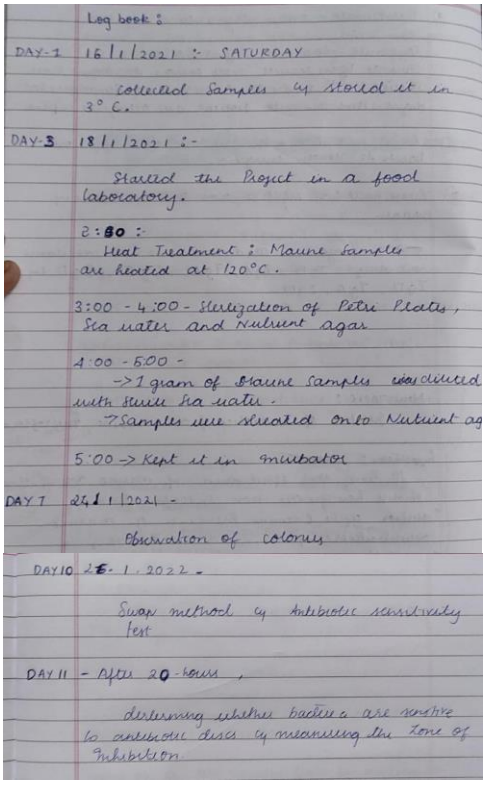
Antibiotic sensitivity test:
Zone of inhibition:
After 20 hours:

After 28 hours:

Table 2

Zone of inhibition	After 20 hours	After 28 hours
Sample 1	4mm	9mm
Sample 2	8mm	13mm
Sample 3	10mm	15mm

Logbook:



4. Discussion

Pour plate method:

The growth was not visible for the first 3 days and later Results showed that the growth of microflora in Nutrient agar plates is high on day 7 and gradually reduces till Day 10.

Antibiotic sensitive test:

- After 20 hours when the samples were removed from the incubator the zone of inhibition was minimum but after 28 hours the antibiotic showed sensitivity to bacteria.
- The isolated marine microflora has mild to moderate antibiotic properties.

Possible errors:

- I think collecting good samples is very much important, we must make sure that we take brackish soil from near the coast of the Bay of Bengal i.e., Marina beach in Chennai if not we might not see the growth of microflora (probably actinomycetes).
- We should also sterile everything properly to get perfect results.

Questions and problems:

No proper growth in Agar Sample 2 IN POUR PLATE METHOD and no proper zone of inhibition after 20 hours.

Data variations:

Samples 1 and 3 had enough colonies but sample 2 had fewer colonies in the POUR PLATE METHOD.

And was able to see a minimum zone of inhibition after 20 hours but gradually increased after 28 hours.

5. Conclusion

My hypothesis was to prove that marine microflora (probably actinomycetes), can be used as an antibiotic. Yes, I have proved that the isolated marine microflora has mild-to-moderate antibiotic properties

Among the multitude of diverse organisms in the marine environment, marine microorganisms stand out as an excellent source of many useful metabolites. Of all the marine microbes, the actinomycetes merit special consideration.

Antibiotics can be made using Marine microflora. The outcome of the study will provide a great breakthrough for fisheries and emerging industries.

This will help to reduce the excessive use of antibiotics which causes drug resistance and Allergic reaction.

6. Future Enhancement

I will do the same project with Starch Casein Agar (SCA) and also prove whether it was the growth of actinomycetes by Biochemical testing. I might also isolate more pure cultures with different types of samples in the Marine.

Acknowledgement

Alhamdulillah! First and foremost, I would like to praise Allah, The Almighty, the Most Gracious, and the Most Merciful for His blessings given to me during my study and in completing this Research.

Next, this paper and the research behind it would not have been possible without the exceptional support of my teacher, Survath Jabeen. Her enthusiasm, knowledge, and exacting attention to detail have been an inspiration and kept my work on track from my first encounter with the collection of samples

to the final draft of this paper. My teacher has always been motivating me to take part in all the competitions taking place and today all the confidence I have is because of her constant support and belief in me.

I would also like to express my special thanks to our principal Mrs. Najia Tahmeem for giving me this wonderful opportunity.

Secondly, I would like to thank, Haniya, my uncles, aunt, and parents who helped me out and supported me throughout the project

Last but not the least, I am extremely grateful to my friend Maseeha Fathima who has always been there by my side.

Without all of them, I could never have completed this task.

References

[1] Handbook of microbiology
[2] https://www.sciencedirect.com/topics/immunology-and-microbiology/antibiotic-disc
[3] Austin, B. 1989. Novel Pharmaceutical compounds from Marine bacteria. J. Appl. Bacteriol. 67: 461-470.
[4] Stonik, V.A., Makarieva, T.N. & Shubina, L.K. Antibiotics from Marine Bacteria. Biochemistry Moscow 85, 1362–1373 (2020).
[5] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71-79.
[6] https://www.sciencedirect.com/topics/immunology-and-microbiology/antibiotic-disc