

ISOLATION AND CHARACTERISATION OF AIR BORNE MICROBES OVER INTERSPACIAL SURFACE OF THE TROPICAL SEA WATER

Submitted in partial fulfillment of the requirements for the award of Bachelor
of science in Microbiology

BY

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL BIO CHEMICAL AND ENGINEERING**

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with “A” grade by NAAC | 12B Status by UGC | Approved by AICTE

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the Bonafide work of **Ms. M. EVANGELIN VINCIA (40300015)** who carried out the project entitled "ISOLATION AND CHARACTERISATION OF AIR BORNE MICROBES OVER INTERSPACIAL SURFACE OF THE TROPICAL SEA WATER" under my supervision from September 2022 to April 2023.

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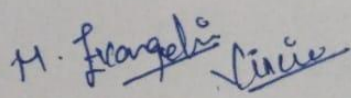
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I **MS. M. EVANGELIN VINCIA (40300015)** here by declare that the Project entitled **"ISOLATION AND CHARACTERISATION OF AIRBORNE MICROBES OVER INTERSPACIAL SURFACE OF THE TROPICAL SEA WATER"** done by me under the guidance of **Dr. C. VALLI NACHIYAR** (Internal guide) and **Dr. L. STANLEY ABRAHAM** (External guide) at **CENTRE FOR OCEAN RESEARCH** in **SATHYABHAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** is submitted in partial fulfillment of the requirements for the award of Bachelor of Microbiology.

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ABSTRACT

This study is aimed to investigate the presence of bacteria in aerosols has been known as air borne microbes. This study proposed monitor the air borne Bacterialcommunity which present in the coastal wetland and costal site. The air samplingwas carried out by the commercial petri Air Sampler. The air sample was collectednear interspatial surface for 30 seconds. Then, the sample collected using NA, MA. TCBS, PDA and incubated for 24 to 48 hours at room temperature. The Colony Forming Unit was recorded. After that, they were isolated and processed for biochemical test. The test has been carried out to identify the bacterial speciesby their biochemical activities. Amylase Enzyme activity was also carried out. Molecular characterization has been done for the identification of isolated strains.The identified bacteria are *Acinetobacter calcoaceticus*, *vibrio sp.*, *Flavobacterium psychrophilum* and *Bacillus sp.* This Study was conducted to compare the Abundance of Airborne Microbes in different Sea Level. Further, this work will help to find out the ocean atmospheric interaction for microbial transmission. The collection of airborne strains will providea basis to greatly increase our understanding of the relationship between bacteriaand marine ecosystem associated with Particulate Matter and marine flora and fauna human health.

KEYWORDS: Airborne microbes, bioaerosols, Air sample, Marine microbes.

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LIST OF ABBREVIATIONS

S. NO	ACRONYM	ABBREVIATIONS
1	NA	Nutrient agar
2	MA	Marine Agar
3	HCL	Hydrochloric acid
4	ml	Milliter
5	EDTA	Ethylene diamine tetra acetic acid
6	TE	Tris ethylene diamine tetra acetic acid
7	G	Gram
8	M	Molar
9	NaCl	Sodium chloride
10	CTAB	Cetyltrimethylammonium bromide
11	SDS	Sodium dodecyl sulfate
12	DNA	Deoxyribonucleic acid
13	RNA	Ribonucleic acid
14	Min	Minutes
15	H	Hours
16	µl	Micro litre
17	nm TCBs	Nano meter
18	PDA	Thiosulfate Citrate Bile Salt Sucrose Agar
19		Potato dextrose agar

CHAPTER 1

1 INTRODUCTION

1.1 EVOLUTION OF MICROBES

often known as a "microscopic organism." To observe them, we must use a microscope. Before its discovery in the seventeenth century, microscopic organisms were speculated to exist for many millennia. The Jains of modern-day India first proposed the existence of microscopic creatures known as nigodas in the sixth century BC. According to legend, these nigodas are thought to be born in groups, live everywhere, including on the bodies of plants, animals, and people, and have a brief existence. The Jain teacher Mahavira claimed that as people eat, breathe, sit, and move around, they significantly damage these nigodas. Many contemporary Jains claim that Mahavira foresaw the presence of germs as it has since been found by science. (Frost *et al.* 2013).

1.2 MICROBIAL ECOSYSTEM

In the biosphere, microbes are everywhere, and their existence always has an impact on the conditions under which they can flourish. When it comes to human measurement or observation, the effects of bacteria on their surroundings can be advantageous, detrimental, or undetectable. The ability of bacteria to recycle the basic components that go into all living things, particularly carbon, oxygen, and nitrogen, has a profound impact on the planet. Primary production is carried out by photosynthetic organisms, which absorb atmospheric carbon dioxide and transform it into organic matter. It is also known as carbon dioxide fixation, and it is responsible for a sizable amount of the organic carbon that is accessible for the creation of cell material. Complex organic compounds degrade through decomposition or biodegradation to various types of carbon that other species can use. Although some manufactured materials, such as plastics, insecticides, and pesticides, are broken down very slowly or not at all, there isn't a naturally occurring organic substance that can't be broken down by some microbe. Organic molecules are eventually converted to carbon dioxide through the microbial metabolic processes of fermentation and respiration, which is then released back into the atmosphere to continue the cycle of primary generation.

Only a few bacteria are capable of biological nitrogen fixation, a process that removes nitrogen from the atmosphere and transforms it into ammonia, which is then used by plants and animals.(Burrows *et al.* 2009).

Additionally, nitrogen fixation replenishes soil nitrogen that has been lost due to agricultural operations. In light of all these advantages, microorganisms also play a significant role in preserving the environment. This chapter primarily focuses on the positive and negative effects that bacteria have on the environment, as well as their function in preserving the environment's quality, health, and sustainability.

1.3 BACTERIAL COMMUNITY

Bacteria are tiny, single-celled organisms with no nucleus and peptidoglycan-based cell walls. Bacteria are the direct ancestors of the first species that have ever lived on Earth. Although some are much larger and some are as microscopic as viruses, most bacteria are far smaller than our own cells. Although they often lack membrane-wrapped organelles like the nucleus, mitochondria, and endoplasmic reticulum, they do have an exterior membrane. Additionally, the majority of bacteria are encased in at least one layer of cell wall. There are many different types of bacteria. Its constituents exist in almost every ecosystem on the earth and come in a variety of shapes, sizes, and functions.

Bacteria in the ocean adapt to be best suited for their environment. Those that are close to the water's surface, like cyanobacteria, tend to be photosynthesizers. It makes sense, then, that the types and numbers of bacteria present at the surface are often tied to location and the season they take advantage of when the temperature and sunlight are just right. Some bacteria feast on dying phytoplankton and only grow in number when there are seasonal algal blooms.(Wager *et al.* 2002)

1.4 TYPES OF MICROBES

Microorganisms, such as bacteria, fungi, and algae, are incredibly small about 1 meter, which is 1,000 times smaller than 1 milli meter. Each and every cell that makes up a microbe is unique. Like human and animal cells, these

cells do not combine to form a larger multicellular creature. Every habitat on Earth has microorganisms, including soils, oceans, ice, and air. Airborne microorganisms are those that can be found in the air and can migrate on their own or in conjunction with dust particles. Dust can be produced as a result of industrial pollutants and car emissions, for example. Airborne microorganisms can be many different types of bacteria, fungi, and algae and can have diverse morphologies, such as being round, rod-shaped, or string-shaped. Think about all the many species. Picture a flock of birds with all of their varying sizes, shapes, and life habits, and then picture that same flock having millions of airborne germs from countless different species, perhaps even thousands. with billions of airborne. In order to survive in the absence of sunlight at greater depths, bacteria evolve special adaptations. As a result, there is generally more bacterial diversity at depth. The open, dark ocean is home to over 70% of all marine microorganisms. In this environment, bacteria are more likely to have flagella, which are swimming tails that help them dodge hungry predators. While the decomposing matter is a fantastic source of nutrients, some people cling to falling heaps of dead particles.

More than 70% of the surface of the Earth is covered by the marine ecosystem. The marine environment offers a variety of habitats for the plant and animal life of the marine aquatic system, which includes everything from seashores to the deep seafloor. Millions of different animals call it home. Seas and oceans on the surface of the Earth are considered to be marine. The marine ecosystem refers to the interactions between living such as plants and animals and non-living elements within the maritime environment. One of our planet's major ecosystems is the marine one. Each living thing in the marine ecosystem depends on the others, forming a fascinating food chain unique to marine life. All of the ecosystem's biotic and abiotic. Over the past ten years, marine ecosystem models have become more complex in response to a growing understanding of the complexity of marine ecosystems and to the demand for adaptable models that can be used for global biogeochemical simulations as well as applications in coastal and estuarine management.

Rapid advances in processing power have made this trend possible. The question of whether or not there will be a point of diminishing returns in the development of these models naturally emerges. In other words, will these models increasing complexity lead to an improvement in their ability to forecast the future, or will it lead to a rise in unpredictability and indeterminacy. Do current ecological modelling techniques have any intrinsic flaws that will ultimately restrict the amount of model complexity that can be useful elements are crucial for preserving correct equilibrium. Quantitative model intercomparison studies, which seek to quantify the impact of increasing model complexity on predictive skill and model portability the latter of which refers to a model's ability to adapt and function properly under various environmental and forcing conditions have been inspired by the desire to answer the first question. Results from these comparisons which so far have concentrated on open ocean systems indicate that, as long as the models are correctly constrained/tuned with the available data, more complicated model formulations can lead to higher prediction skill and more portability. (Alyssa *et al.* 2021)

There are numerous ways to study natural systems, and these approaches frequently emphasise different kinds of patterns. A quantitative, typically statistical, analysis of community structure can be developed from the natural fascination with the wide variety of species present in any site of land or water. This analysis will show similarities in the relative abundance of species within various communities and will define terms like niches. On the other hand, the complexity and variety of potential environmental adaptations can be revealed by studying the social or sexual behaviour of a given species in ever-increasing detail. Indirect evidence of how species interact with one another can be found in the general organisation of populations and in an individual's actions when foraging, reproducing, or evading predators. The study of energy flow in food webs, where relationships between species or groups of species are the main issue, falls between these two techniques. Although this approach seems to be fairly neutral, it implicitly makes the assumption that energy is the standard unit for measuring other variables. The connotations of the word efficiency, when applied to the development of people or the comparison of communities, highlight the assumed generality.

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The term "marine microbe" refers to a wide range of microorganisms, including viruses, bacteria, and archaea. These organisms are incredibly tiny, with a volume of only 1/8000 that of a human cell and a diameter of roughly 1/100 that of a human hair. One millilitre of seawater can support up to a million of them.

The diversity and spread of microorganisms, especially in the deep sea, remain under-sampled and unknown despite being present everywhere on Earth in your tummy, dirt, and the oceans. The Earth's processing factories for biological, geological, and chemical biogeochemical interactions are known to be microorganisms. The majority of marine microorganisms live in diverse, intricate, and challenging to study communities that are highly organised and dynamic. They make up more than 98 percent of the biomass in the ocean and are as variable as the surroundings they call home.

These organisms can survive in almost any setting and obtain energy from a multitude of sources, including solar radiation and chemosynthesis. They perform a variety of functions in the marine environment, including serving as the foundation of the food chain, regulating a large portion of the flow of marine energy and nutrients, and being crucial to the wellbeing of the ocean. (Scott *et al.* 2012)

Ocean change may be detected through the rapid evolution of marine microbial populations in response to environmental changes. In fact, marine bacteria may serve as the marine environment's canary in the coal mine. They also act as the ocean's primary change agents. Therefore, it is crucial to gather fundamental knowledge about these microorganisms so that any future modifications can be compared to it.

CHAPTER 2

2. LITERATURE SURVEY

2.1. MICROBES

Microbes are common and have received a lot more research in recent years. In this review, we provide thorough information on airborne microorganisms that often come from liquid-air and soil-air interfaces in soil and water. Additionally, a variety of variables, including unique meteorological phenomena like dust storms, can have an impact on the stability of the microbial community structure in bioaerosols. These events even have the potential to spread alien viruses and harm people. These habitats are typical for that location and have an impact on the type of airborne microorganisms that interact with them. For instance, the quantity and concentration of airborne microorganisms are significantly influenced by climatic variables, haze, and foggy days. However, due to the high concentration of chemical contaminants in particulate matter, which microorganisms in the atmosphere are attracted to. Over the past 20 years, major changes have been made to the social lives of microorganisms. Prior to recent discoveries, it was believed that bacteria and other microorganisms had relatively independent, unicellular lives devoid of the cooperative behaviours that have sparked such curiosity in mammals, birds, and insects. Bacteria's social interactions and organisation are essential to their ecological success. But we have only recently started researching these hidden groups. (Zhengsheng *et al.* 2018)

2.2. AIRBORNE MICROBES

The arithmetic topography, and environmental variables of different locales all affect the load of airborne microorganisms. The diversity of airborne microorganisms can change over time and space because the atmosphere is a heterogeneous environment, just like any other microbial home. bacteria typically enter the atmosphere from anthropogenic soil and plants and natural sources, but their survival and dispersal rely on the bacteria' cell structures and weather patterns. Increased infections were linked to low temperatures,

while airborne bacterial diversity was positively correlated with temperature, atmospheric pressure, and wind speed. However, high winds during pollution episodes reduced local airborne bacterial concentrations, which could have enhanced aerosol atomization processes on soil and water surfaces. About 55% of the changes in airborne bacterial community architectures were explained by the chemical compositions of metal elements and ions. (Romano *et al.* 2020).

2.3. BIOGEOCHEMICAL

In both maritime and atmospheric habitats, microbes are pervasive. They make up over 70% of all marine biomass in the ocean¹ and are essential to biogeochemical cycles such as the carbon, nitrogen, and sulphur cycles. A significant portion of atmospheric bioaerosols is made up by the microbial community, but little is known about the variables influencing their diversity, abundance, and function in the atmosphere, as well as the microbial exchange between the two environments. Understanding the ocean's function as a source and sink of microorganisms as well as the movement of airborne bacteria will help us better understand how terrestrial communities interact with the ocean and the spatial distribution and diversity of microorganisms. Overland, a variety of sources, from man-made to natural ecosystems, produce airborne microorganisms. Bacteria can be produced at the ocean's surface by wind-driven processes, locally released as sea spray aerosols over the oceans, or transported great distances from terrestrial sources into the marine atmosphere. It has been hypothesised that all airborne bacteria can move globally and may grow in any habitat with favourable environmental circumstances due to their aerodynamic sizes. So it stands to reason that a significant portion of the local marine microbiome would be present in the airborne bacterial community aloft in a particular maritime environment. (Karl *et al.* 2009)

2.4. MARINE ECOSYSTEM

On Earth, the marine ecosystem covers more than 70% of the land site. From seashores to the deep ocean, the marine environment offers a wide variety of habitat for the marine aquatic system's flora and wildlife. Millions of species live there. Because microbial operations must be understood at the scale of the individual bacterium, microbiological oceanography is a field that is trapped between scales. On the one hand, we wish to understand the cumulative impact of microbial processes on how the ocean functions as a biogeochemical system. It is then necessary to scale up the local effects of microorganisms on biogeochemical cycles in order to make reliable predictions about how marine ecosystems across the entire ocean might react to climatic change. Aquatic environments with high dissolved salt concentrations, such as those in or near the ocean, are known as marine ecosystems. The unique biotic living and abiotic non-living components of marine ecosystems are what set them apart. Biologic impacts include things like plants, animals, and bacteria; abiotic influences include things like the quantity of sunlight that falls on a site, how much oxygen and nutrients are dissolved in the water, how close the site is to land, depth, and temperature. The activities of this large aquatic ecosystem have significantly changed the flows of growth-restricting nutrients from the landscape to receiving waters. Unfortunately, these fertiliser inputs have had a significant deleterious impact on surface water quality all across the world. In both freshwater and coastal marine settings, this review explores how eutrophication affects the biomass and species composition of algae. The interplay of living plants and animals and non-living inanimate species within the marine culture is known as the marine ecosystem. A marine culture food chain is formed by the interdependence of each living thing in the marine ecosystem. Freshwater environments, which contain less salt, are distinct from marine ecosystems. (Epstein *et al.* 1993).

2.5. SOURCE OF MICROBES

Microorganisms are naturally present in soil, water and in air, and therefore exterior surface of plants and animals are contaminated with a variety of microorganism. No active airborne processes exist for bacteria. They are

spread on dust particles that have been disturbed by physical forces and in tiny water droplets produced by any procedure that results in the development of an aerosol. This process is experienced by many actinomycetes, particularly those belonging to the genus *Streptococcus*. Microorganisms mainly travel through air. Compared to soil or water, they are scarcer in the air. Both good non-pathogenic and bad pathogenic bacteria are present in small amounts in the air, although the majority of the germs are good. Studying the microflora in the air can be divided into two categories: indoor and outdoor microflora. Atmospheric temperature, humidity low and high relative humidity induce the death of most microorganisms, and air current are environmental elements that affect air microflora. 37 different genera of bacteria were found in the air, with *Bacillus*, *Micrococcus*, and *Staphylococcus* accounting for the majority of them. The germs from plant or animal surfaces may enter the air via air currents. These microorganisms could be plant or animal pathogens or commensals. According to studies, plant pathogenic microbes can travel very far through the air. (Marius et al. 2012).

2.6. BACTERIAL ATMOSPHERE

Bacteria are present everywhere in the atmosphere, with concentrations over land generally surpassing. Numerous studies have suggested that the presence of bacteria in the atmosphere may have an effect on the formation of clouds, the chemistry of the atmosphere, and the biogeography of microbes. To evaluate these assertions, one must have a thorough understanding of bacterial concentrations and distributions in the atmosphere. This review focuses on previously reported measurements of the levels of total and culturable bacteria in atmospheric aerosol. We go over emission mechanisms, the effects of weather, measurement methods, and determined bacteria concentrations. We propose typical values and ranges for the mean concentration in the near-surface air of nine natural ecosystems and three human-influenced land types based on the research we have studied. We talk about the gaps in the present understanding of bacterial concentrations in the air, such as the dearth of trustworthy, long-term observations of the overall microbial concentrations in many places and the dearth of emission flux measurement.

Bioaerosols are microorganisms such as bacteria, fungi, and viruses linked to atmospheric particulate matter. According to reports, bioaerosols can make up to 25% of the atmospheric aerosols. Additionally, it is well recognised that chemical pollutants have a significant role in the development of airborne particulate matter, which can lead to unfavourable human diseases, particularly respiratory conditions. Airborne microorganisms embedded in atmospheric particles, in addition to chemical pollutants, have drawn increasing attention in recent years due to the evidence for their role in the atmospheric environment and possible effects on human health, agricultural productivity, and ecosystem stability.(Reed *et al.* 2016)

The dispersal of air current and droplets is attributed to the spread of microorganisms from liquid systems or soil to the atmosphere, which is the best explanation for the presence of some specific microorganisms present in completely different environments. Additionally, soil and marine environments are the principal sources of airborne microbes, followed by processing plants where microbe dispersal is dependent on the function of the microorganism. For instance, numerous studies have demonstrated that airborne microorganisms share many traits and structural elements with marine and soil microbes. Droplet dispersion in facilities such as composting, waste disposal, and sewage treatment, which primarily depend on bacteria and fungi for decomposition and disposal, also helps to raise the number of microorganisms in the atmosphere. In bioaerosols, there are numerous and numerous species of bacteria and fungi. However, the quantity of airborne microorganisms tends to decline on particularly cloudy days because of the enriched atmospheric contaminants in particulate materials.

2.7. ADVANTAGES OF AIRBORNE MICROBES

The health, biogeochemical cycling, and the global climate system may all be significantly influenced by airborne microbes. Dust storms are an atmospheric occurrence that carry a lot of the microorganisms linked to dust particles through the Earth's atmosphere. Due to its ability to transport them across great distances, air current is also crucial in the dissemination of microbes. The particles containing germs have a tendency to settle in quiet air. However, a

slight breeze can maintain them in suspension for a very long time. (Angeles *et al.* 2018)

2.8 DISADVANTAGE OF AIRBORNE MICROBES

In the harsh environment of the atmosphere, organisms are exposed to low temperatures and intense radiation. Numerous microorganisms found there exhibit resistance or have systems of adaptation built to tolerate the harsh environment. Humans' upper and lower respiratory tracts are exposed to a range of vapours and airborne particles through inhalation. Disease can be brought on by airborne transmission of harmful microbes to humans from the environment, animals, or other people. (Stetzenbach *et al.* 2009) As the lung is more prone to illness than the digestive system, inhalation is a significant method of exposure.

CHAPTER 3

3. AIM AND SCOPE

3.1 AIM OF THE PROJECT

The aim of the project is that

- To isolate and characterize the airborne microbes over the interspatial tropical surface of the sea water.

3.2 SCOPE OF THE PROJECT

The scope of the project is

- The collection of the airborne strains will provide a basis to greatly increase our understanding of the relationship between bacteria and fungi associated with particular matter and human health.

3.3 OBJECTIVES

- Isolation of air borne microbes from the interspatial surface of the tropical sea water in different locations like Salt-pans, Marshy lands, Brackish water and Sea Shore.
- To characterize the isolation by the biochemical characterization.
- To test whether the isolated bacteria produce amalyse enzyme.
- To determine the sensitivity or resistance of the collected bacterialsample by disc diffusion method.
- Molecular characterization of the predominant microbial species.

CHAPTER 4

4 MATERIALS AND METHODOLOGY

4.1 MATERIALS REQUIRED

- Petri dishes
- Test tubes
- Conical flask
- Slides
- Beaker
- Inoculation loop
- Centrifuge tubes

4.2 CLEANING OF GLASS WARES

Firstly, the empty glassware was rinsed in the water. Then, the glassware was immersed in the dilution of soap solution for 30 mins. Finally, the glass wares were washed in the distilled water. The glass wares were kept in hot air oven for drying.

4.3 STERILIZATION OF GLASSWARE:

The glass wares were sterilized in the autoclave at 121 degrees Celsius for 15 minutes.

4.4 MEDIA

The basic media like nutrient agar, marine agar and the selective media like Thiosulfate citrate bile salts sucrose agar. Then the potato dextrose agar was prepared for fungal culture.

4.4.1 PREPARATION OF THE MEDIA

Each media is prepared for 100ml

1. PREPARATION OF NUTRIENT AGAR

Nutrient agar = 2.8g

Agar = 1.5g

2. PREPARATION OF MARINE AGAR

Marine agar = 5.525g

Agar = 1.5g

3. PREPARATION OF TCBS AGAR

Thiosulfate citrate bile salt sucrose agar = 8.908g

Agar = 1.5g

4. PREPARATION OF PDA AGAR

Potato dextrose agar = 3.9g

Agar = 1.5g

4.5 SAMPLE COLLECTION

The bacterial samples were collected using Commercial Petri Air Sampler for 30 seconds from the interspatial surface of the tropical sea water in different locations like salt-pans, marshy lands, brackish water and sea shore across different sites in Tamil Nadu like Marakkanam, Paramankeni, Alamparai and kovalam. The strains were isolated in the agar plate.



Figure 4.5.1 Sample collection In Marakkanam Salt pans



Figure 4.5.2. Sample collection
In Marshy land



Figure 4.5.3. Sample collection in Kovalam



Figure 4.5.4. Sample collection in Paramankeni
Brackish water

4.6 ISOLATION AND SUBCULTURING OF THE COLLECTED SAMPLES

4.6.1 ISOLATION OF THE SAMPLE

The sample were collected and incubated for 48 hours at room temperature.

4.6.2 SUBCULTURING OF THE SAMPLES

After incubation for 48 hours, the grown colonies were sub cultured in agar slants.



Figure 4.6.2.1 Subcultured Slants

4.7 STAINING TECHNIQUES

Gram staining is a method frequently used to distinguish between two sizable groups of bacteria based on the distinct components of their cell walls, such as gram-positive and gram-negative bacteria.

4.7.1 PROCEDURE

- First, the bacterial culture smear was prepared
- It was stained with the primary stain crystal violet for 30 seconds and rinsed in distilled water for 2 seconds.
- Few drops of Gram's iodine were added for 1 minute and rinsed in distilled water.
- Add a few drops of decolorizer and rinse it with distilled water immediately.
- Next, the counter stain safranin was added for 30 to 60 seconds and rinsed with distilled water.

- The slides were dried and observed under microscope.

4.8 BIOCHEMICAL CHARACTERIZATION

By separating various bacterial species based on their metabolic activities, biochemical tests are performed to identify specific bacterial species.

The performed biochemical tests are:

- INDOLE
- METHYL RED
- VOGES PROSKAUER
- CITRATE UTILIZATION
- CATALASE

4.7.1 INDOLE TEST

To determine the ability of microorganism to degrade the amino acid tryptophan.

PROCEDURE:

- ☐ First, the tryptophan broth was prepared and sterilized
- ☐ The bacterial stain was inoculated in the broth and incubated for 24 hours at room temperature.
- ☐ After 24 hours incubation, Kovacs reagent was added into it.
- ☐ The colour changes were observed.

4.7.2 METHYL RED

To determine which fermentation pathway is used to utilize glucose.

PROCEDURE:

- The glucose phosphate broth was prepared and sterilized.
- The bacterial culture was inoculated in the broth and incubated for 24 hours at room temperature.
- After 24 hours incubation, few drops of methyl red reagent was added to it. The colour changes were observed

4.7.3 VOGES PROSKAUER

To determine the ability of organisms to produce non-acidic or neutral end product.

PREPARATION OF REAGENT

- **Barritt's reagent A:**

*6grams Alpha- naphtholin in 100ml of 95% of ethanol were added kept in magnetic stirrer for 5 minutes.

- **Barritt's reagent B:**

*40grams of potassium hydroxide in 100ml of distilled water were added and kept in magnetic stirrer until it dissolves

PROCEDURE

- The glucose phosphate broth was prepared and sterilized.
- The bacterial culture was inoculated in the broth and incubated for 24 hours at room temperature.
- After 24 hours incubation, few drops of Barritt's-A and Barritt's-B reagent was added to it.
- The colour changes were observed.

4.7.4 CITRATE UTILIZATION TEST

The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source.

PROCEDURE:

- Simmons citrate agar was prepared and sterilized.
- The bacterial culture was streaked in the agar slant and kept for incubation for 24 hours.
- After incubation, the appearance of slant culture will appear blue then it is positive.
- No colour change indicates negative result.

4.7.5 CATALASE TEST

To determine the ability of some microorganism to degrade hydrogen peroxide by producing the enzyme catalase.

PROCEDURE

- Using sterile technique, inoculate each experimental organism into nutrient agar slant.
- Incubate tubes for 24 – 48 hours at 37°C.
- Allow three or four drops of the 3% hydrogen peroxide to overflow over the entire surface of each slant culture.
- Observe for the formation of or absence of bubbling or foaming.

4.6 MOTILITY

Hanging drop method is the traditional method for examining the cell motility and morphology by taking the living microorganisms from the liquid media.

PROCEDURE

- ☐ The clean coverslip was taken and the corner of it by using a toothpick or earbud.
- ☐ After that, a loopful of the microbial suspension where placed on the centre of the prepared coverslip.
- ☐ Then, a clean or grease-free cavity glass slide was taken and turn it upside down. The cavity slide was placed in a manner that the circular concavity should be towards the drop of fluid over the coverslip.
- ☐ Again, the cavity glass slide was placed in such a manner that the microbial specimen will find inverted over the concave depression or observed hanging from the coverslip.
- ☐ Then the prepared culture was placed slide on the slide holder of the light microscope and adjust the just in a way that the edge of drop must be visible through the eyepiece.
- ☐ 10X objective lens were used and closed the diaphragm. Then, slowly the coarse was adjusted to focus knob accordingly until it was observed a curve that crossing the field of view, in which one half shows the edge of the drop and the other half shows the edge of concave depression.
- ☐ The fine focus knob was adjusted by looking to the specimen over the slide through the eyepiece, until the edge of the drop
 - ☐ It appeared as a thick dark line.
- ☐ *Then, look onto each side of the line and focus onto the particulate matter in the fluid suspension. The cells may vary in colour, size and shape.
- ☐ *After that, 40X objective lens was used for higher resolution and also turn on the diaphragm lever. Then, adjust the light in order to maximize the visibility of the microorganisms.

- After all this set-up, carefully observe the specimen and note down the microscopic properties like the shape, colour, motility of the specimen placed on the glass slide. All the cells will show Brownian movement, but few may possess true motility.

4.7 ENZYMES ACTIVITY TEST

This is aimed to identify out the isolated bacteria that can produce amylase enzyme.

PREPARATION OF STARCH MEDIA

- Soluble starch =2g/200ml.
- Beef extract =0.6g/200ml.
- Agar =3g/200ml.

PROCEDURE

- The starch agar was prepared and sterilized.
- The sterilized agar was poured in the sterilized Petri dish.
- After the solidification, the inoculum was spreaded in the starch agar and incubated for 7 days at 37°C.
- Next, the plates were flooded with Gram's iodine.
- Any colonies with formation of clear zone around the colonies were subcultured in liquid broth and streaked at least five times to obtain pure isolates as confirmed by Gram staining and 1000X light. Microscopic observation.

4.8 ANTIMICROBIAL SUSCEPTIBILITY TEST

The Kirby-Bauer test for antibiotic susceptibility also called the disc diffusion test. Tetracycline disc was used in this technique. Tetracyclines are

categorized as broad-spectrum antibiotics that suppress protein synthesis.

PROCEDURE:

Mueller Hinton agar was prepared and sterilized.

- Sterilize the site with disinfectant and open burner before performing the test.
- A sterile cotton swab is dipped into the inoculum and remove excess medium by pressing the swab onto the wall of the tube.
- Swab the surface site of the plate completely by rotating the plate. This technique is called lawn culture or carpet culture.
- Allow the plates to dry for 5 minutes so that the medium absorbs the inoculum properly.
- Firstly, sterilize the forceps with alcohol before picking up antibiotic discs.
- Discs should be placed at a distance of 24mm.
- Incubate the plate upside down for 24 hours at 37°C.

4.12 MOLECULAR CHARACTERISTICS

Molecular characterization by 16s rRNA gene sequence analysis is a cost-effective tool to identify simpler to complex microbial strains at species level.

- Marine agar kovalam site [KA] (1)
- Marine agar Kovalam site [KA] (2)
- Marine agar Kovalam site [KA] (3)
- Marine agar Mangrove site [MG]
(1)
- Marine agar paramankeni site
[PA] (1)

- Marine agar Paramankeni site [AA]
(2)
- Marine agar Alamaparai site [AA]
(1)

BUFFER USED FOR THE EXTRACTION

PREPARATION OF EXTRACTION BUFFER

1000Mm Tris HCL = 10ml (from 1M)

100mM EDTA = 20ml (from 0.5M)

100mM Sodium phosphate = 10ml

Nacl = 30ml (from 5M)

Miliq water = 30ml

PROCEDURE

1. Bacterial pellets were mixed with 50 microlitre Extraction buffer (100mM Tris HCl, 100mM EDTA, PH, 8, 100mM sodium phosphate, bPH.8.0 1.5M Nacl) and centrifuged at 10000rpm for 5 mins at 25°C.
2. The supernatant was discarded and 500microlitre extraction buffer 50microlitre, CTAB, 50%SDS and 10microlitre of proteinase K (20 mg/L) was added mixed well and incubate at 55°C for 2hr.
3. Samples were centrifuged for 10 min at 10,000rpm and the supernatant was transferred to a new tube.
4. The resulting supernatant were pooled and mixed with an equal volume of chloroform: iso- amyl alcohol (24:1) and centrifuged at 10,000rpm 25°C - 20 mins.

5. To the aqueous phase, 600microlitre of 100% chilled ethanol / isopropanol was added + 40% sodium acetate (7M) chilled and invert the tube several times and themixture was kept at 4°C overnight.
6. Centrifuged at 10,000rpm for 20mins.
7. Then the pellet was washed with 70% chilled alcohol and centrifuged at 10000rpm for 5mins. The process was repeated for one more time.
8. The DNA pellet was washed with 90% Ethanol. Centrifuged at 10,000 for 5mins.
9. The DNA pellet was dried and resuspended in TE Buffer.
10. Bacterial pellets were mixed with 50 microlitre Extraction buffer (100mM Tris HCl,100mMEDTA, PH, 8, 100mM sodium phosphate, bPH.8.0 1.5M Nacl) and centrifuged at 10000rpm for 5 mins at 25°C.
11. The supernatant was discarded and 500microlitre extraction buffer 50microlitre, CTAB, 50%SDS and 10microlitre of proteinase K (20 mg/L) was added mixed well and incubate at 55°C for 2hr.
12. Samples were centrifuged for 10 min at 10,000rpm and the supernatant was transferred to a new tube.
13. The resulting supernatant were pooled and mixed with an equal volume of chloroform: iso- amyl alcohol (24:1) and centrifuged at 10,000rpm 25°C - 20 mins.
14. To the aqueous phase, 600microlitre of 100% chilled ethanol / isopropanol was added + 40% sodium acetate (7M) chilled and invert the tube several times and themixture was kept at 4°C overnight.
15. Centrifuged at 10,000rpm for 20mins.

16. Then the pellet was washed with 70% chilled alcohol and centrifuged at 10000rpm for 5mins. The process was repeated for one more time.
17. The DNA pellet was washed with 90% Ethanol. Centrifuged at 10,000 for 5mins.
18. The DNA pellet was dried and resuspended in TE Buffer.

5 CHAPTER

5.RESULT AND DISSCUSION

5.1 COLONY FORMING UNIT IN NUTRIENT AGAR AND IN MARINE AGAR

Estimation of the number of viable bacteria in a sample. A colony is a cluster of bacteria growing together. Here, the colony forming unit of the bacterial sample which were collected and isolated in Nutrient Agar and in marine agar. It was observed for 10 days. The site Alamparai has more number of colonies when compared to other sites. The colony forming unit were figured below in bar diagram from figure 5.1.1 to 5.1.5.

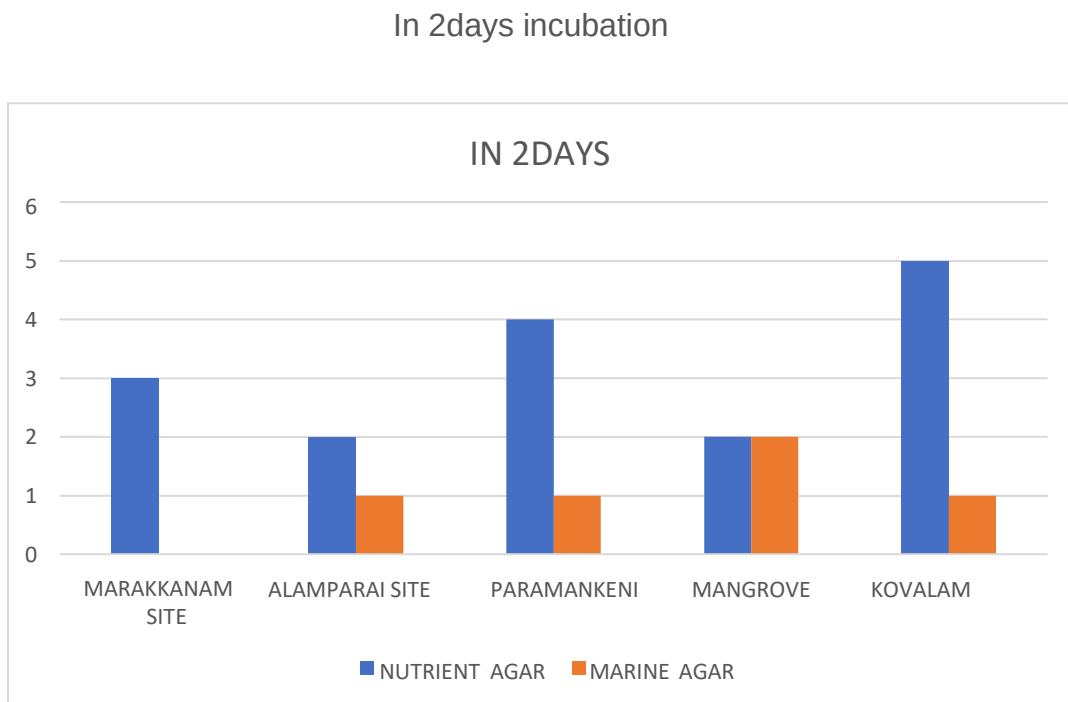


Figure 5.1.1 Colony forming unit in nutrient agar and in marine agar

In 4days incubation

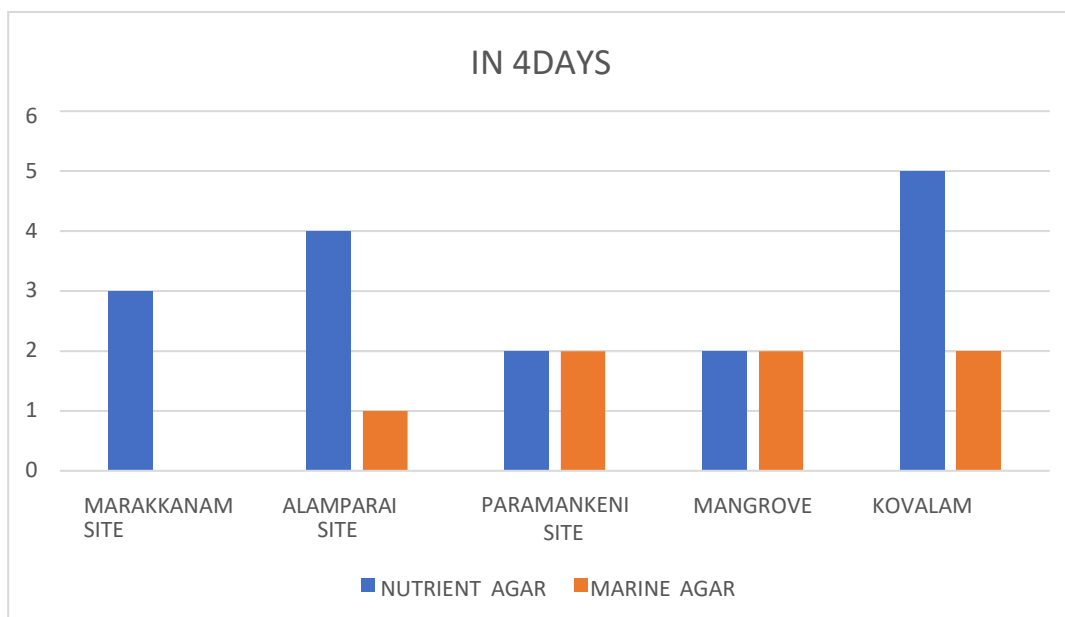


Figure 5.1.2 Colony Forming unit in nutrient agar and in marine agar

In 6days incubation

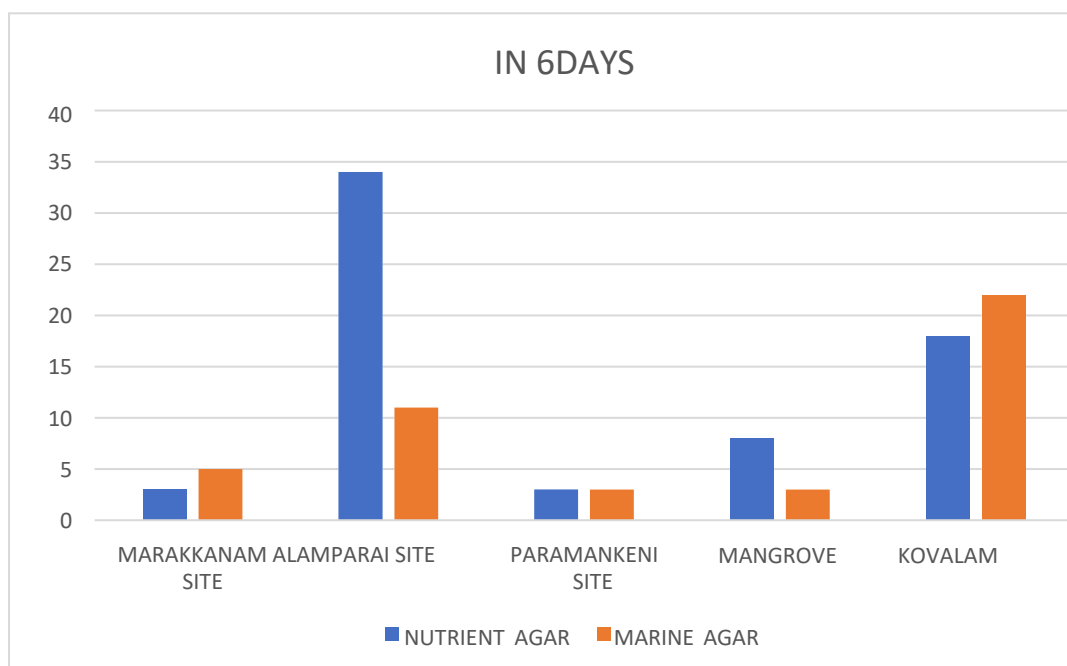


Figure 5.1.3. Colony forming unit in nutrient agar and in marine agar
In 6days incubation

In 8days incubation

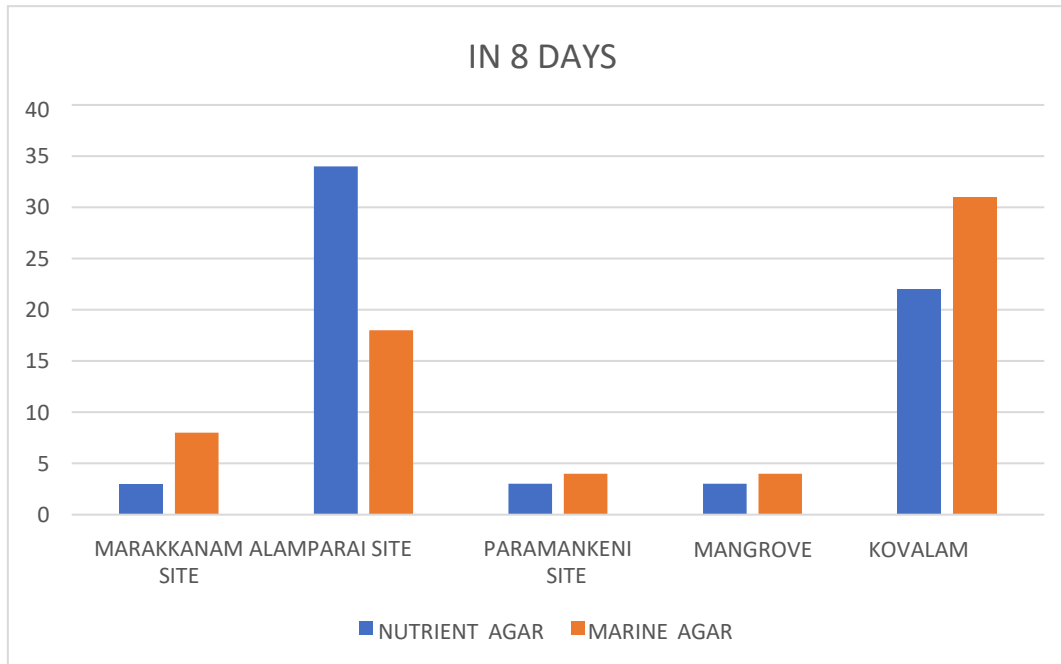


Figure 5.1.4. Colony forming unit in nutrient agar and in marine agar

In 10days incubation

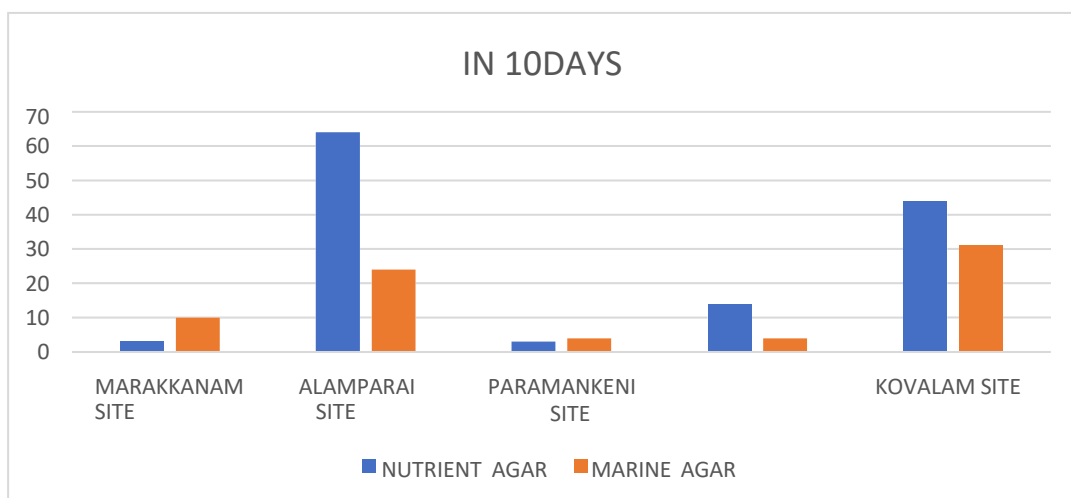


Figure 5.1.5. Colony forming unit in nutrient and in marine agar

In incubation 10 days

5.1 COLONY APPEARANCE IN AGAR PLATES

LOCATION AND STRAINS NUMBER	MORPHOLOGY IN NUTRIENT AGAR PLATES
Paramankeni site 1	white colour colony, Creamy appearance.
Paramankeni site 2	pale yellow colour
Paramankeni site 3	yellow colour colony, smooth colony.
Marakkanam site1	pale white colour, sticky colony.
Marakkanam site 2	pale yellow colour colony.
Mangrove site 1	pale yellow colour colony.
Mangrove site 3	Transparent colony.
Alamparai site 1	pale white colony, mucoid.
Alamparai site 2	Orange colour colony, smooth colony.
Alamparai site 3	pale yellow colour, mucoid colony.
Alamparai site 4	white colour colony.
Alamparai site 5	pale yellow colour, dry colony.
Kovalam site 1	yellow colour colony.
Kovalam site 2	white colour colony, sticky colony.
Kovalam site 3	pale white colour colony, mucoid colony.
Kovalam site 4	pale white, dry rough colony.
Kovalam site 5	pale yellow colour, smooth colony.
Kovalam site 6	white colour colony, smooth colony.

Table 5.2.1. Colony morphology in the nutrient agar plates

LOCATION AND STRAINS NUMBER	MORPHOLOGY IN MARINE AGAR PLATES
Paramankeni site1	pale white smooth colony
Paramankeni site 2	brownish smooth colony
Paramankeni site 3	white smooth colony
Alamparai site1	white dry colony
Alamparai site 2	yellow smooth colony
Alamparai site 3	orange smooth colony
Kovalam site 1	orange dry colony
Kovalam site 2	white smooth colony
Kovalam site 3	white smooth colony
Mangrove site 1	White colour colony, dry colony
Mangrove site 2	white colour, creamy colony
Mangrove site 3	yellow colour, creamy colony

Table 5.2.2. Colony morphology in the marine agar plates

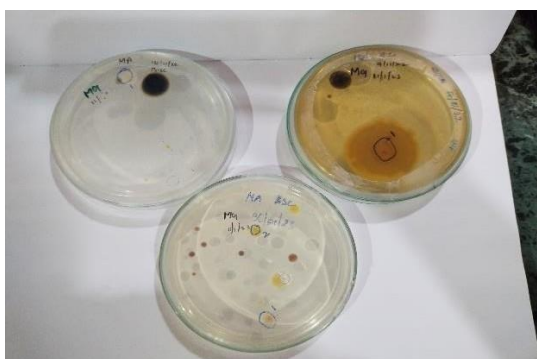


Figure 5.2.1 Mangrove site

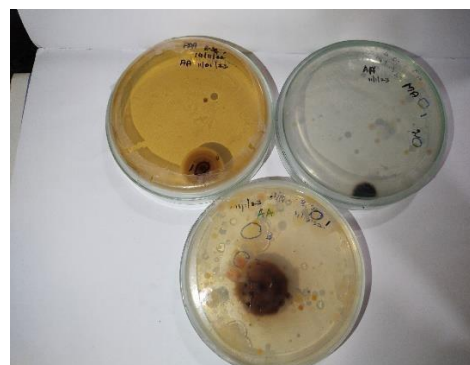


Figure5.2.2 Alamparai site



Figure 5.2.3 Kovalam site



Figure 5.2.4. Marakkanam site

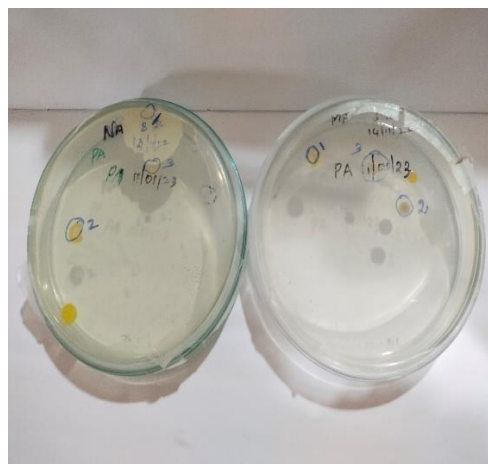


Figure 5.2.5. Paramankeni site

DISCUSSION

The colony morphology of the isolated bacteria in nutrient agar and in the marine agar has many white colour colonies. So, it is similar to the related publication. (Abirami *et al.* 2019)

5.2 GRAM STAINING

Gram staining differentiates bacteria by the chemical and physical properties of their cell walls. Gram-positive cells have a thick layer of peptidoglycan in the cell wall that retains the primary stain, crystal violet. It tells about the type of bacterial cell.

IN NUTRIENT AGAR PLATES

LOCATION AND STRAINS NUMBER	GRAM POSITIVE OR GRAM NEGATIVE	MICROSCOPIC MORPHOLOGY IN NUTRIENT AGAR
Paramankeni site 1	Gram positive	Rod
Paramankeni site 2	Gram positive	Cocci
Paramankeni site 3	Gram negative	Rod
Alamparai site 1	Gram positive	Cocci
Alamparai site 2	Gram positive	Cocci in cluster
Alamparai site 3	Gram negative	Rod
Alamparai site 4	Gram positive	Cocci

Alamparai site 5	Gram positive	Cocci
Kovalam site 1	Gram positive	Rod
Kovalam site 2	Gram positive	Rod
Kovalam site 3	Gram negative	Rod
Kovalam site 4	Gram positive	Cocci
Kovalam site 5	Gram positive	Cocci
Kovalam site 6	Gram positive	Cocci
Marakkanam site 1	Gram positive	Rod
Marakkanam site 2	Gram positiv	Cocci. grape like structure
Mangrove site 1	Gram positive	Cocci
Mangrove site 3	Gram positive	Cocci

Table 5.3.1. Gram staining results in nutrient agar

IN MARINE AGAR PLATES

LOCATION AND STRAINS NUMBER	GRAM POSITIVE OR GRAMNEGATIVE	MICROSCOPIC MORPHOLOGY IN MARINE AGAR
Paramankeni site1	Gram positive	Cocci
Paramankeni site 2	Gram positive	Cocci
Paramankeni site 3	Gram positive	Cocci
Alamparai site 1	Gram positive	Rod
Alamparai site 2	Gram positive	Cocci
Alamparai site 3	Gram positive	Rod
Kovalam site 1	Gram negative	Cocci
Kovalam site 2	Gram positive	Rod
Kovalam site 3	Gram positive	Rod
Mangrove site 1	Gram positive	Rod
Mangrove site 2	Gram positive	Cocci
Mangrove site 3	Gram negative	Rod

Table 5.3.2. Gram staining results in marine agar

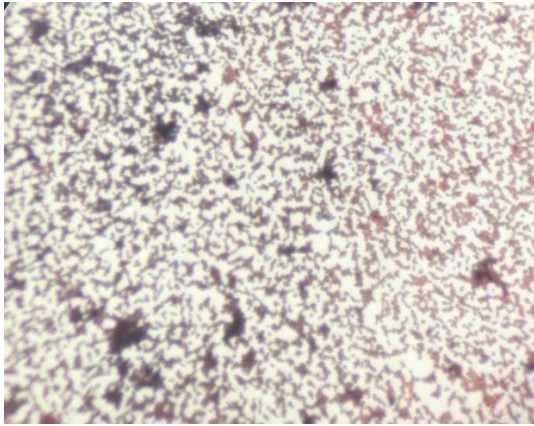


Figure 5.3.1. Mangrove site [MG] (2)

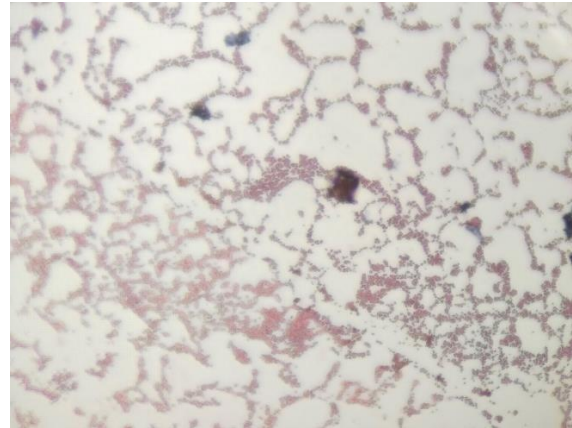


Figure 5.3.2 Paramankeni site [PA](1)

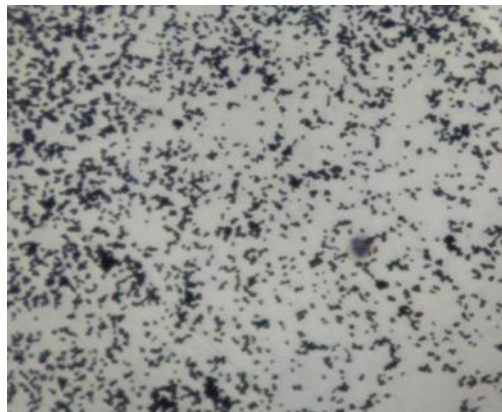


Figure 5.3.3 Marakkannam site [MA] (4)

5.4 BIOCHEMICAL CHARACTERIZATION

5.4.1 INDOLE TEST

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. It is used as part of the IMViC procedures. Tryptophan is an amino acid that can be oxidized by some bacteria to form three major end products: indole, pyruvic acid, and ammonia.

IN NUTRIENT AGAR STRAINS

ISOLATED SAMPLES	Original	Duplicate	Duplicate
Alamparai site 1	-	-	-
Alamparai site 2	-	-	-
Alamparai site 3	-	-	-
Alamparai site 4	-	-	-
Alamparai site 5	-	-	-
Kovalam site 1	-	-	-
Kovalam site 2	-	-	-
Kovalam site 3	-	-	-
Kovalam site 4	-	-	-
Kovalam site 5	-	-	-
Kovalam site 6	-	-	-
Paramankeni site 1	-	-	-
Paramankeni site 2	-	-	-
Paramankeni site 3	-	-	-
Marakkanam site 1	-	-	-

Marakkanam site 2	-	-	-
Mangrove site 1	-	-	-
Mangrove site 2	-	-	-

Table 5.4.1.1 indole test results for nutrient agar strains

IN MARINE AGAR STRAINS

ISOLTED SAMPLES	Original	Duplicate	Duplicate
Alamparai site 1	-	-	-
Alamparai site 2	-	-	-
Alamparai site 3	-	-	-
Kovalam site 1	-	-	-
Kovalam site 2	-	-	-
Kovalam site 3	-	-	-
Paramankeni site 1	-	-	-
Paramankeni site 2	-	-	-
Paramankeni site 3	-	-	-
Mangrove site 1	-	-	-
Mangrove site 2	-	-	-
Mangrove site 3	-	-	-

Table 5.4.1.2 indole test results for marine agar strains



Figure 5.4.1.1 Nutrient agar of all strains in indole test



Figure 5.4.1.2 Marine agar of all strains in indole Test

5.4.2 METHYL RED TEST

The test like Methyl red and Voges Proskauer both are used to determine which fermentation pathway is used to utilize glucose. In the mixed acid fermentation pathway, glucose is fermented and produces several organic acids .

IN NUTRIENT AGAR STRAINS

ISOLTED SAMPLES	Original	Duplicate	Duplicate
Alamparai site 1	-	-	-
Alamparai site 2	+	+	+
Alamparai site 3	-	-	-
Alamparai site 4	-	-	-
Alamparai site 5	-	-	-
Kovalam site 1	-	-	-
Kovalam site 2	-	-	-
Kovalam site 3	-	-	-
Kovalam site 4	-	-	-
Kovalam site 5	-	-	-
Kovalam site 6	-	-	-
Paramankeni site 1	-	-	-
Paramankeni site 2	-	-	-
Paramankeni site 3	-	-	-
Marakkanam site 1	-	-	-
Marakkanam site 2	-	-	-
Mangrove site 1	-	-	-
Mangrove site 2	-	-	-

Table 5.4.2.1 methyl red test results for nutrient agar strains

IN MARINE AGAR STRAINS

ISOLTED SAMPLES	Original	Duplicate	Duplicate
------------------------	-----------------	------------------	------------------

Alamparai site 1	-	-	-
Alamparai site 2	-	-	-
Alamparai site 3	-	-	-
Kovalam site 1	-	-	-
Kovalam site 2	+	+	+
Kovalam site 3	-	-	-
Paramankeni site 1	-	-	-
Paramankeni site 2	-	-	-
Paramankeni site 3	-	-	-
Mangrove site 1	-	-	-
Mangrove site 2	-	-	-
Mangrove site 3	-	-	-

Table 5.4.2.2 Methyl red test results for marine agar strains



Figure 5.4.2.1 All strains of nutrient agar

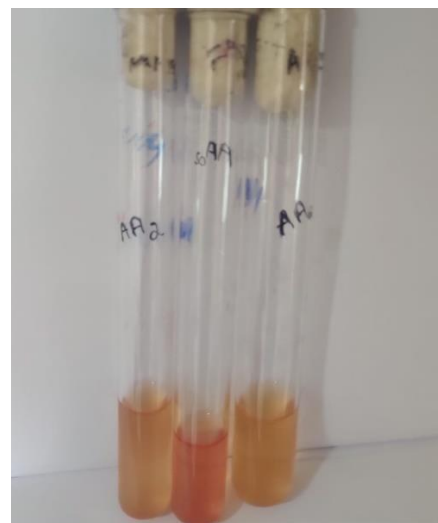


Figure 5.4.2.2 Positive strains.



Figure 5.4.2.3 All strains of marine agar.

5.4.3 VOGES PROSKAUER TEST

This Voges Proskauer test is used to determine which fermentation pathway is used to utilize glucose. In the mixed acid fermentation pathway, glucose is fermented and produces several organic acids .

IN NUTRIENT AGAR STRAINS

ISOLATED SAMPLES	Original	Duplicate	Duplicate
Alamparai site 1	-	-	-
Alamparai site 2	-	-	-
Alamparai site 3	-	-	-
Alamparai site 4	-	-	-
Alamparai site 5	-	-	-
Kovalam site 1	-	-	-
Kovalam site 2	-	-	-
Kovalam site 3	-	-	-
Kovalam site 4	+	+	+
Kovalam site 5	-	-	-
Kovalam site 6	-	-	-
Paramankeni site1	-	-	-
Paramankeni site 2	-	-	-
Paramankeni site 3	-	-	-
Marakkanam site 1	-	-	-
Marakkanam site 2	-	-	-
Mangrove site 1	-	-	-
Mangrove site 2	-	-	-

Table 5.4.3.1 Voges Proskauer test results for nutrient agar strains

IN MARINE AGAR STRAINS

ISOLATED SAMPLES	Original	Duplicate	Duplicate

Kovalam site 2	-	-	-
Kovalam site 3	-	-	-
Paramankeni site 1	-	-	-
Paramankeni site 2	-	-	-
Paramankeni site 3	-	-	-
Mangrove site 1	-	-	-
Mangrove site 2	-	-	-
Mangrove site 3	-	-	-
Alamparai site 1	-	-	-
Alamparai site 2	-	-	-
Alamparai site 3	-	-	-
Kovalam site 1	-	-	-

Table 5.4.3.2 Voges Proskauer test results for marine agar strains



Figure 5.4.3.1

All strains of marine agar.



Figure 5.4.3.2

All strains of nutrient agar.



Figure 5.4.3.3
Control Samples for Voges proskauer Test

5.4.4 CITRATE UTILIZATION TEST

The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. Citrate is a natural compound which can be utilized as a carbon and energy source by many bacterial species.

IN NUTRIENT AGAR STRAINS

ISOLTED SAMPLES	Original	Duplicate	Duplicate
Alamparai site 1	-	-	-
Alamparai site 2	-	-	-
Alamparai site 3	-	-	-
Alamparai site 4	-	-	-
Alamparai site 5	-	-	-
Kovalam site 1	-	-	-
Kovalam site 2	-	-	-
Kovalam site 3	-	-	-
Kovalam site 4	-	-	-
Kovalam site 5	-	-	-
Kovalam site 6	+	+	+
Paramankeni site 1	+	+	+
Paramankeni site 2	+	+	+
Paramankeni site 3	-	-	-
Marakkanam site 1	-	-	-
Marakkanam site 2	-	-	-
Mangrove site 1	-	-	-
Mangrove site 2	-	-	-

Table 5.4.4.1 Citrate utilization test results for nutrient agar strains

IN MARINE AGAR STRAINS

ISOLTED SAMPLES	Original	Duplicate	Duplicate

Kovalam site 1	-	-	-
Kovalam site 2	-	-	-
Kovalam site 3	-	-	-
Paramankeni site1	-	-	-
Paramankeni site 2	-	-	-
Paramankeni site 3	+	+	+
Mangrove site 1	-	-	-
Mangrove site 2	-	-	-
Mangrove site 3	-	-	-
Alamparai site 1	-	-	-
Alamparai site 2	-	-	-
Alamparai site 3	-	-	-

Table 5.4.4.2 Citrate utilization test results for marine agar strains

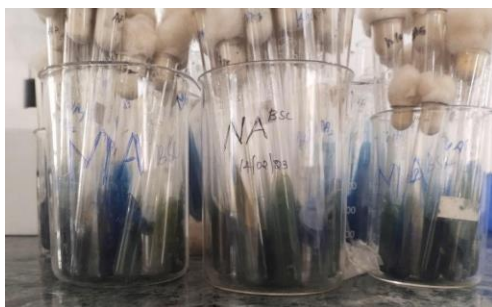


Figure 5.4.4.1
Nutrient agar strains.



Figure 5.4.4.2
Marine agar strains.

5.4.5 CATALASE TEST

IN NUTRIENT AGAR STRAINS

ISOLTED SAMPLES	HYDROGEN PEROXIDE
Alamparai site 1	-
Alamparai site 2	+
Alamparai site 3	-
Alamparai site 4	+
Alamparai site 5	+
Kovalam site 1	-
Kovalam site 2	+
Kovalam site 3	+
Kovalam site 4	-
Kovalam site 5	-
Kovalam site 6	-
Paramankeni site 1	-
Paramankeni site 2	-
Paramankeni site 3	+
Marakkanam site 1	-
Marakkanam site 2	+
Mangrove site 1	+
Mangrove site 2	-

Table 5.4.5.1 Catalase test results for nutrient agar strains

IN MARINE AGAR STRAINS

ISOLTED SAMPLES	HYDROGEN PEROXIDE
Alamparai site 1	+
Alamparai site 2	+
Alamparai site 3	+
Kovalam site 1	+

Kovalam site 3	-
Paramankeni site 1	-
Paramankeni site 2	-
Paramankeni site 3	-
Mangrove site 1	+
Mangrove site 2	+
Mangrove site 3	-
Kovalam site 2	+

Table 5.4.5.2 Catalase test results for marine agar strains



Figure 5.4.5.1 Nutrient agar Mangrove site [MG] (1)



Figure 5.4.5.2 Nutrient agar Marakkanam site [MA] (2)

5.5 MOTILITY

- The result is negative when there is no movement, or there is the presence of the non-motile organism.

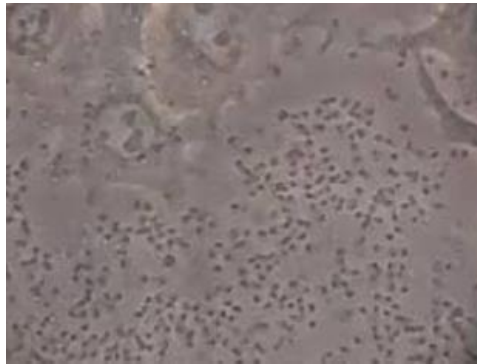


Figure 5.5.1 Marine Kovalam site [KA] (3)

5.6 ENZYME ACTIVITY OF ANALYSE

- All the strains show negative result for amylase enzyme activity because there is no clear zone is appeared. So, the isolated strains do not produce amylase enzyme.

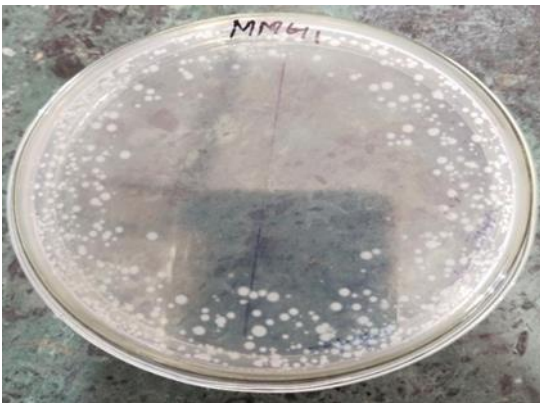


Figure 5.6.1

Marine Mangrove site [MG] (1)

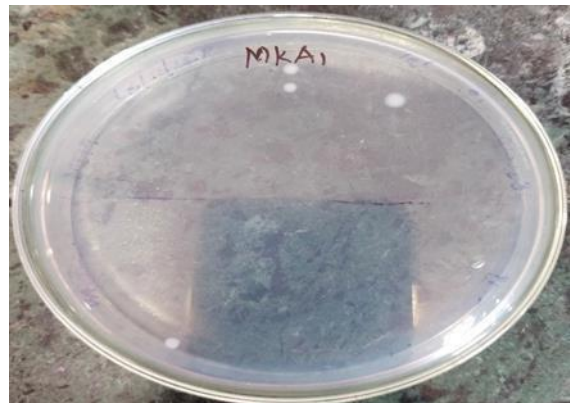


Figure 5.6.2

Marine Kovalam site [KA] (1)



Figure 5.6.3
Marine Kovalam site [KA] (3)



Figure 5.6.4
Marine agar mangrove site [MG] (1)

5.7 DISC DIFFUSION ANTIBIOTIC TEST

- In this regard, we are isolating microbes and screening for antimicrobial resistance activity using tetracycline antibiotic disc.
- Nutrient agar Mangrove site [MG] (3), Marine agar Mangrove site [MG] (1), Nutrient agar Kovalam site [KA] (2), Nutrient agar Kovalam site [KA] (4). These strains have zone of Inhibition in the tetracycline antibiotic disc.



Figure 5.7.1
Marine agar Kovalam site [KA] (2)



Figure 5.7.2
Nutrient agar Kovalam site [KA] (2)

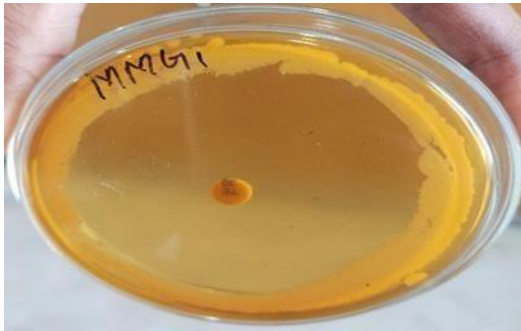


Figure 5.7.3
Marine agar Mangrove site [MG] (1)



Figure 5.7.4
Marine agar Mangrove site [MG] (1)

5.8 MOLECULAR CHARACTERIZATION

- Isolation of bacteria and morphological characterization of bacteria. A total seven different bacterial colonies were isolated from marine water sample on the basis of unique colonial characteristics on nutrient agar and marine agar. All the seven bacterial isolates exhibited different morphological and colonial characteristics.



Figure 5.8.1
Strains for DNA isolation

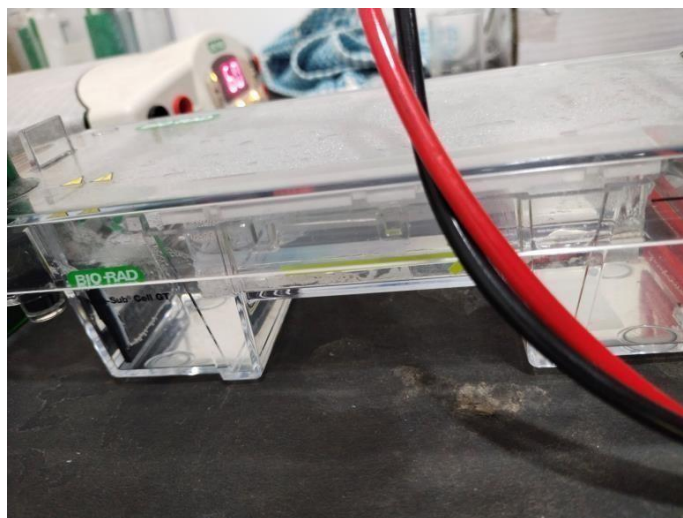
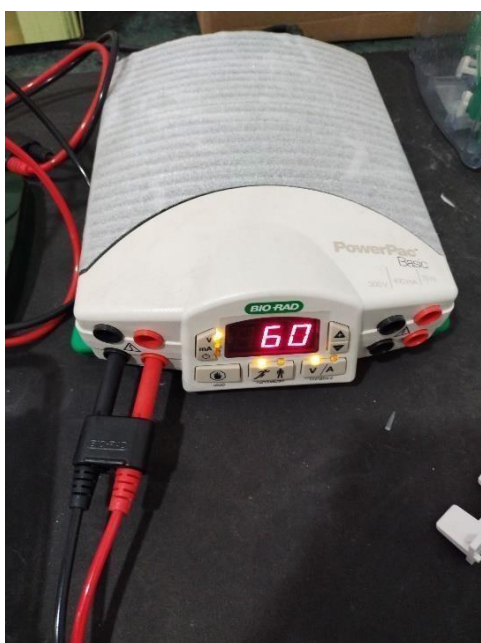


Figure 5.8.2 Agarose gel electrophoresis

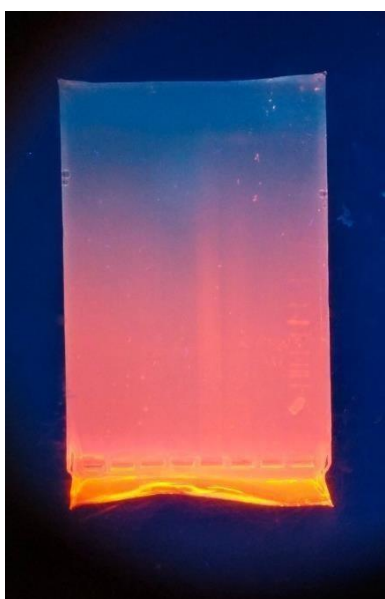


Figure 5.8.3

Bands on agarose gel electrophoresis



Figure 5.8.4

PCR

ESTIMATION OF DNA USING NANO DROP

Samples	DNA concentration	280/260	260/230
Marine agar Alamparai site (1)	814.9µg/ml	1.75	0.48
Marine agar Paramankeni site (1)	249.2µg/ml	2.14	0.35
Marine agar Paramankeni site (2)	586.1µg/ml	2.02	0.36
Marine agar Kovalam site (1)	1317.0µg/ml	1.87	0.99
Marine agar Kovalam site (2)	183.2µg/ml	2.04	0.84
Marine agar Kovalam site (3)	631.1µg/ml	2.20	0.67
Marine agar Mangrove site (1)	501.8µg/ml	2.08	0.90

Table 5.8.1 Estimation of DNA using nano drop

5.8.1 RESULTS OF 16s rRNA SEQUENCE

Marine agar mangrove site (3) MMG3

Acinetobacter calcoaceticus

16 S rRNA partial sequence

atcacttagc ggcggacggg tgagtaatgc ttaggaatct gcctattagt gggggacaac
atttcgaaag gaatgctaata accgcatatc tcctacggga gaaagcaggg gatcttcgga
ccttgcgcta atagatgagc ctaagtcgga ttagctagtt ggtggggtaa aggcctacca
aggcgacgat ctgtagcggg tctgagagga tgatccgcca cactgggact gagacacggc
ccagactcct acgggaggga gcagtgggga atattggaca atgggcgcaa gcctgatcca
gccatgccgc gtgtgtgaag aaggccttat ggttgtaaag cactttaagc gaggaggagg
ctactgaagt taataccttc agatagtga cggtactcgc agaataagca ccggctaact
ctgtgccagc agccgcggta atacagaggg tgcaagcgtt aatcggattt actgggcgta
aggcggctaa ttaagtcaaa tgtgaaatcc ccgagcttaa ctgggaatt caggattaga
gcattcgata ctggtagct agagtgtggg agaggatggt agaattccag gtgtagcgg
gaaatgcgta gagatctgga ggaataccga tggcgaaggc agccatctgg cctaactg
acgtgaggt gcgaaagcat ggggagcaaa caggattaga taccctggta gtccatgccg
taaacgatgt ctactagccg ttggggcctt tgaggcttta gtggcgagc taacgcgata
agtagaccgc ctggggagta cggtcgcaag actaaaactc aaatgaattg acggggggccc
gcacaagcgg tggagcatgt ggtttaattc gatgcaacgc gaagaacctt acctggcctt
gacatagtaa gaactttcca gagatggatt ggtgccttcg ggaacttaca tacaggtgct
gcatggctgt cgtcagctcg tctcgtgaga tgttgggta agtcccgcga cgagcgcaac
cctttcctt attgccagc gagtaatgtc gggaaactta aggatactgc cagtgacaaa
ctggaggaag gcggggacga cgtcaagtca tcatggccct tacggccagg
gtacacacg tgctacaatg gtcggtacaa aggggtgcta cctagcgata
ggatgctaata ctcaaaaagc

Marine kovalam area(3) MKA3

Vibrio sp.

16 S rRNA partial sequence

gacgggtgag taatgcctgg gaaattgccc tgatgtgggg gataaccatt ggaaacgatg
gctaataccg cataatagct tcggctcaaa gagggggacc ttcgggcctc tcgctgcagg
atatgcccag gtgggattag ctagttggtg aggtaatggc tcaccaaggc gacgatccct
agctggtctg agaggatgat cagccacact ggaactgaga cacgggtccag actcctacgg
gaggcagcag tggggaatat tgcacaatgg gcgcaagcct gatgcagcca tgccgcgtgt
gtgaagaagg ccttcgggtt gtaaagcact ttcagcagtg aggaagggtg tgtcgtaat
agcggcatca ttgacgta gctgcagaag aagcaccggc taactccgtg ccagcagccg
cggaataacg gagggggcga gcgtaatcg gaattactgg gcgtaaagcg catgcgggtg
nttagttaag tcagatgtga aagcccgggg ctaccctcg gaactgcatt tgaaactggc
tgactagaga actgtagagg ggggtagaat ttcagggtga gcggtgaaat gcgtagagat
ctgaaggaat accgggtggc gaaggcgccc cctgnncaga tactgacact canatgcgaa
agnnnngggg agcaaacagg atttgatacc ctggtagtcc acgccgtaaa cgatgtctac
ttggagggtg tggccctgag ccgtggattt aggagctaac gcgtaagta gaccgcctgg
ggagtaccgt cgcaagatta aaactcaaat gaattgacgg gggcccgcac aagcggtgga
gcatgtggtt taattcgatg caccgcgaag aaccttacct actctgaca tccagagAAC
ttagcagaga tgctttggtg ccttcgggaa ctctgagaca ggtgctgcat ggctgtcgtc
agctcgtgtt gtgaaatgtt gggtaagtc ccgcaacgag cgcaaccctt atccttgttt
ggacgacgtc aagtcacat ggcccttacg agtagggcta cacacgtgct acaatggcgc
atacagaggg cgccaactt gcgaaagtga gcgaatcca aaaagtgcgt cgtagtccgg
attggagtct gcaactcgac tccatgaagt cggaatcgct agtaatcgtg gatcagaatg

Marine agar alamparia area (1) MAA1

Flavobacterium psychrophilum

16 S rRNA partial sequence

cccagagaaa ttggattaa tacctcatag tatagtgagt tggcatcaac acactattaa
agtcacaacg gtaaaagatg agcatgcgtn cccattagct agttggaag gtaacggctt
accaaggcaa cgatgggtag gggcctgag agggagatcc cccacactgg tactgagaca
cggaccagac tcctacggga ggcagcagtg aggaatattg gtcaatgggc gcaagcctga
accagccatg ccgcgtgcag gatgacggtc ctatggattg taaactgctt ttgcacagga
agaaacaytc ggtcgtgacc gagcttgacg gtactgtgag aataaggatc ggctaactcc
gtgccagcag ccgcggtaat acggaggatc caagcggtat ccggaatcat tgggtttaa
gggtccgtag gcggccagat aagtcagtgg tgaaagccca tcgctcaacg atggaacggc
cattgatact gtttggttg aattatttg aagtaactag aatatgtagt gtagcgggta
aatgcttaga gattacatgg aataccaatt gcgaaggcag gttactacaa attgattgac
gctgatggac gaaagcgtgg gtagcgaaca ggattagata ccctggtagt ccacgccgta
aacgatggat actagctgtt gggagcaatc tcagtggcta agcgaaagtg ataagtatcc
cacctgggga gtacgttcgc aagaatgaaa ctcaaaggaa ttgacggggg cccgcacaag
cgggtggagca tgtggtttaa ttcgatgata cgcgaggaac cttaccaagg cttaaattga
gattgaccgg ttggaaaca gactttcgc aagacaattt acaagggtgct gcatggtgt
cgtcagctcg tgccgtgagg tgtcaggtta agtcctataa cgagcgcaac ccctgttgtt
agttgccagc gagtcatgtc gggaactcta gcaagactgc cagtgtaaac tgtgaggaag
gtggggatga cgtcaaatca tcacggccct tacgccttgg gctacacacg

Marine paramankeni area(1) MPA1
Bacillus sp.

16 S rRNA partial sequence

tgcaagtcga gcgaatggat tgagagcttg ctctcaagaa gttagcggcg gacgggtgag
taacacgtgg gtaacctgcc cataagactg ggataactcc gggaaaccgg ggctaatacc
ggataacatt ttgaactgca tggttcgaaa ttgaaaggcg gcttcggctg tcacttatgg
agccgacctg agaggggtgat cggccacact gggactgaga cacggcccag actcctacgg
gaggcagcag tagggaatct tccgcaatgg acgaaagtct gacggagcaa cgccgcgtga
gtgatgaagg ctttcgggtc gtaaaactct gttgttaggg aagaacaagt gctagttaa
taagctggca ccttgacggg acctaaccag aaagccacgg ctaactacgt gccagcagcc
gcgtaatac gtaggtggca agcgttatcc ggaattattg ggcgtaaagc gcgcgcaggt
ggtttcttaa gtctgatgtg aaagcccacg gctcaaccgt ggaggggtcat tggaaactgg
gagactgag tgcagaagag gaaagtggaa ttccatgtgt agcggtgaaa tgcgtagaga
aagcgtgggg agcagacagg attagatacc ctggtagtcc acgccgtaaa cgatgagtgc
taagtgttag agggtttccg cccttagtg ctgaagttaa cgcattaagc actccgcctg
gggagtacgg ccgcaaggct gaaactcaaa ggaattgacg ggggcccgcg caagcgggtg
agcatgtggt ttaattcgaa gcaacgcgaa gaaccttacc aggtcttgac atcctctgaa
aactctagag atagggcttc tccttcggga gcagagtgac aggtgggtgca
tggttgtcgt

CHAPTER 6

CONCLUSION

The air samples were collected from the various ecosystem such as Salt-pans, brackish water, seashore and marshy land of various locations like Marakkanam-Viluppuram district [Latitude 12.218553°N and Longitude 79.970417°E], Mangrove are near Marakkanam- Villupuram district [Latitude 12.232379°N and Longitude 79.978691°E], kovalam-Chengalpattu district [Latitude 12.786012°N and Longitude 80.255119°E], Alamparai-Viluppuram district [Latitude 12.2573°N and Longitude 79.9973°E], Paramankeni-Chengalpattu district [Latitude 12.353949°N and Longitude 80.060133°E] using Commerical Petri Air Sampler and Nutrient agar and Marine agar plates were used for isolation of bacteria.

The media sampled from above locations were incubated at room temperature and morphologically different isolates were subcultured in corresponding agar plates. The total count in these plates were enumerated at one day interval for 10 days. The samples collected at Alamparai site has shown maximum total count, followed by samples collected at Kovalam, Mangrove area, Marakkanam and then Paramankeni. The predominant isolates from these sites were characterized by using biochemical and molecular characterization. The gram staining was carried out and most of the isolates were of Gram positive.

The biochemical characterization was done to identify the bacterial species by differentiating them on the basis of biochemical activities. IMViC test has been done. All the isolated bacterial samples are negative to indole test. Only two strains are positive in the methyl red test. For Voges Proskauer, the nutrient agar kovalam site 4 only shows positive result. Few strains of the nutrient agar and the marine agar are shown positive result. Some strains of the nutrient agar and the marine agar were observed as the positive result.

Molecular characterization was done using sequencing V 4 region by 16 S rRNA sequencing for analysing the phenotypically variants among the isolates. The identified bacteria are *Acinetobacter calcoaceticus*, *vibrio sp.*, *flavobacterium psychrophilum* and *bacillus sp.*

The predominant isolates were evaluation for their enzyme activity with special reference to Amylase enzyme production. The isolates have shown negative enzyme activity. In addition, the antimicrobial sensitivity for standard antibiotic Tetacycline was also evaluated. About the Nutrient agar Mangrove site [MG] (3), Marine agar Mangrove site [MG] (1), Nutrient agar Kovalam site [KA] (2) and Nutrient agar Kovalam site [KA] (4) strains are sensitive and other strains are resistant.

The present study has inferred that bacteria are transmitted in line with wind current and they depend on parameters like Salinity, temperature and anthropogenic activity. This study paves a base for impact of saline environmental locations on distribution of microbes.

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