STUDIES ON BACTERIAL COMMUNITIES ASSOCIATED WITH CORALS

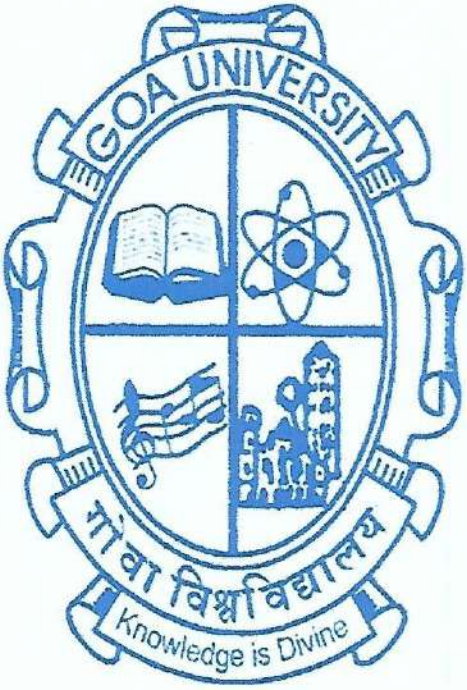
Dissertation thesis submitted to

Goa University

In partial fulfillment of the requirement for the degree of

Master of Science in Marine Biotechnology

for the academic year 2022-23



By

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled "Metagenomic studies on Bacterial Communities associated with Corals", is based on the results of work carried out by me in the Discipline of Marine Biotechnology at the School of Biological Sciences and Biotechnology, Goa University under the supervision of Prof. Sanjeev Ghadi and the same has not been submitted elsewhere for the award of a degree or diploma by me. Furthermore, I understand that Goa University or its authorities will not be responsible for the correctness of the observations, experimental and other findings given in the dissertation.

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COMPLETION CERTIFICATE

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ACKNOWLEDGEMENT

George Lucas once said, “The best way to pursue happiness is to help other people. Nothing else will make you happier”. This dissertation has been an extremely fulfilling and enriching experience for me. I have gained knowledge I thought was beyond my scope and I would like to extend my appreciation to all the people involved in making this project a successful one.

First and foremost, I would like to thank Department of Biotechnology, Government of India, for making this project a success by providing the funds which were necessary in carrying out the desired work.

I would like to extend my gratitude towards Zoological Survey Of India, Marine Biology Regional Centre and Indian Oil Corporation, for providing the opportunity for work under the ZSI - ICOL Coral Translocation by providing permission and funds for sampling.

I owe a indebted and deepest appreciation to Dr. Chowdula Satyaranarayan, Scientist C, Zoological Survey Of India, Marine Biology Regional Centre, for encouragement and support, without whom this project would have been possible. I am inspired by all his dedication in conserving corals and occupational contentment. He has positively steered me towards my goals and I will carry his values in my work throughout my career in conserving coral.

I would like to extend my gratitude towards School of Biological Sciences and Biotechnology, Goa University, for providing all the necessary equipment in a timely and orderly manner.

I would like to express my gratitude and heartfelt appreciation to my dissertation guide, Dr. Sanjeev C. Ghadi, SBSB, for supporting my interest and nurturing. His guidance,constructive suggestion, and valuable advice helped me with all my work.

I would also like to express my heartfelt gratitude to Dr. Savita Kerkar, Dean, SBSB for providing the cultures which were crucial in carrying out the experiments. I would also like to thank all my faculty members- , Dr. Dhermendra Kumar Tiwari, Dr. Meghanath Prabhu, Dr. Sanika Samant, Ms. Dviti Mapari, Dr. Samantha Fernandes, Ms. Snesha U. Bhomkar for providing me with the words of encouragement, advice and motivation whenever I needed it.

I would like to extend my sincere thanks to Ms. Devika Jadhav, Ms. Elaine Da Costa, Ms. Veda Manerikar, Ms. Hetika Kotecha and to all Research Scholars.

I would like to offer a big token of appreciation to our laboratory assistant Mrs. Sandhya, Ms. Jaya for their prompt actions, laboratory attendants Mr. Sameer, Mr. Ashish, Mr. Serrao for providing me with the necessary requirements.

I would love to thank my classmates and friends for providing me with a stable and friendly environment where I could carry out my work in peace. Himanshi, Pooja, Samuel, Kanhaiya. Krupa, Sanjay, Shubham, Manas, Abhranil, Viraj, Lovely, Divyanshi, Adrija, Aditi, Archita, Geetanjali, Aayushi, Ritu, Sanjana, Mayuri have been my pillars of support during this project. They have provided me with a memorable, fun filled experience at the laboratory.

I would like to thank my parents for believing in me and my aspirations. They have always pushed me to me achieve my goals and it was largely due to their words of encouragement that I was able to achieve what I have. Their constant support, blessings and love have been a source of motivation for me.

Lastly, I would like to thank Almighty God for giving me the patience and perseverance which was required to carry out this project.

Thank you all!

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ABBREVIATIONS

ZMA	Zobel Marine Agar
ASW	Artificial Sea Water
GOK	Gulf Of Kutch
GOM	Gulf Of Munnar
CAB	Coral Associated Bacteria
psu	Parts per salinity unit
ppt	Parts per thousand
HGT	Horizontal Gene Transfer
UVR	Ultra Voilet Radiation
SML	Surface Mucous Layer
µL	Microliter
mL	Milliliter
°C	Degrees Centigrade
DNA	Deoxyribonucleic acid
g	Gram
h	Hour
PCR	Polymerase Chain Reaction
rpm	Revolutions per minute

CHAPTER I

INTRODUCTION

INTRODUCTION

1.1 Coral Reefs

Corals are hard calcareous organisms, classified under Phylum Cnidaria which includes jellyfish , hydra and sea anemone having stinging cells in common to protect themselves and hunt for food (Satyanarayana,ch etal., 2009). Cnidarians are predominant marine species which are also found in freshwater. There are two main types of Corals - Hard Corals. These are also known as Reef forming or Hermatypic Corals.). The second type of Corals are Soft Corals These are the Non-Reef forming type, also known as Ahermatypic Corals. Reef forming corals are classified under Order Scleractinia (Stony Corals). Examples of Hard Corals are Staghorn Corals, Table Corals, Brain Corals, Elkhorn Corals, Great Star Coral, Leafy Corals, Branching Corals, and Honeycomb Corals (Venkataraman., 2011).

There are three types of Reefs formed by Corals.These are Fringing Reef, Barrier Reef and Atoll Reef . Coral Reef zones are divided based on the location of the reefs and characteristics such as depth, light intensity, wave energy, temperature and water chemistry. The most profound type of Reefs are the Fringing Reefs These reefs grow outward from the coastlines of islands and continents. A Fringing Reef can be divided into three zones : Reef flat, Reef crest and Fore reef (Venkataraman., 2011).

Reef building coral ecosystems are essential and highly productive ecosystems in the coastal zone. They are responsible for protecting the shores from natural disasters like floods, storms, tsunamis and coastal erosions. They act as breeding and nursery grounds for a wide variety of fishes, gastropods, echinoderms, polycheaetes, ascidians, crustaceans and even mammals. 25% of the world fishery resource is obtained from the coral reef environments even though it occupies only 2% of the Earth’s surface.. (Satyanarayana,ch etal., 2009).

1.2 Coral biology

Corals are distinguished due to their polyp structures. These structures are soft-bodied with multiple tentacles and form a hard skeletal structure with Calcium carbonate. Polyps are the building blocks of a Coral colony. A single polyp which grows and divides to form a massive colony. The body of a hard coral polyp has smooth and tubular structures with a free horizontal oral disc above and an attached basal disc below. The oral disc with the opening at the centre, opens into the polyp interior which is used as mouth and anus (Satyanarayana,ch et al., 2009). Stomadaeum is a small oesophagus like connection between the mouth and the interior gastrovascular cavity. The mouth opening is surrounded by the rings of tubular retractile structures called Tentacles. The tentacles are mostly extend during night times and retract immediately with simple touch (Satynarayana,ch etal., 2009). Retraction is effected through muscular partitions in the body called mesenteries which are extended radially and distributed evenly in its tubular body structure. Coral tissues have an outer epidermal layer, which secretes the skeleton, and an inner tissue layer termed the gastrodermis. Between these tissues lies a thin, fibrous matrix termed the mesogloea. Species specific Endosymbionts reside in the Gastroderm cell layer covering the coelenteron or stomach of the polyp (Venkatraman, et al.., 2003).

1.3 Geo-morphology of GOK Coral Reef

Gujarat, a western state in India, has a long coastline of about 1675 kms with the Arabian Sea between 20 ° N to 24 ° N latitudes. Around 42 islands of coral reefs are present in Gulf of Kutch (GOK). The maximum depth of GOK is 122 m (401 feet). Major coral reefs in Gujarat are observed mostly along the Jamnagar coast of GOK. Coral reefs in the GOK are the northern most in the Indian Ocean. However these coral reefs are geographically isolated (Unnikrishnan .,2023)

.

Wave climate at the head of the GOK is dominated during South West monsoon period (June to September).The Kutch region in general falls within the arid to hyper arid belt of western India. In general, the maximum salinity ranges from 36.6 psu (practical salinity unit similar to parts per thousand or ppt) at the mouth to 45.5 psu near the head of GOK (Vethamony, et al., 2007).

GOK and Gulf of Mannar (GOM) have more similar species in comparison to species similarity between GOK and Lakshadweep region as well as GOK and Andaman & Nicobar islands. This is because of the similar sea environment conditions of GOK and GOM (Jyoti .,et al 2016).

1.4 GOK Coral Diversity

Due to the harsh environmental conditions of GOK , very less diversity of corals are known to inhabit this region. Dominant coral species in GOK are *Favia favus, Turbinaria peltata, Goniastrea pectinata, Montipora sp, Porites sp, Symphyllia radians, Acanthastrea hilae* (Satyanarayana,ch etal.,2009).

The adaptability level of corals in GOK is due to changing tides, currents and anthropogenic disturbances. Morphological changes due to changing environmental conditions are seen in some coral species which are challenging for the taxonomist in identifying species. Species like *Favia favu*s and *Turbinaria pelatata* are two dominant species growing on mobile rocks and Gastropod shells.*Symphyllia radians, Goniastrea pectinata* and *Goniopora sp.*, are preferhabitat of Rock pools which never go dry and also grow to massive proportions to remain submerged in sub-tidal areas

1.5 Coral Associated Bacteria

Coral reefs are threatened by raising ocean temperatures, harmful UV radiations and ocean acidification. Warm waters lead to bleaching of corals, stripping away photosynthetic microbes residing in their tissues (Hung N. Dao.,et al 2021). In some Coral Reef habitats, there are gradual temperature changes. Scientists have discovered that adaptation and subsequent tolerance for higher temperature among corals with various study methods such as larval and juvenile heat stress experiments, molecular identification of symbionts, quantitative genetic breeding experiments . The heat resilience of corals is mainly dependent on the endosymbiotic partners in responses to the stress (K. M. Quigley and M. J. H. van Oppen 2022).

Endosymbiotic microbes associate with the corals are species specific. These also mitigate on the geographical location of Reefs. Isolation, culture and genomic studies of these gives us a better knowledge about the more resilience microbial pattern associated with Corals (Rodrigo .,et al, 2020).

Coral associated bacterial communities are found in the mucus layer, tissue and Calcium carbonate skeleton of the corals (Li et al., 2014). Microbes in the Calcium carbonate skeletons outnumber their counterparts in mucous and tissue (Marcelino and verbruggen, 2017; Ricci et al., 2019). Bacteria residing in the corals have the potential to form biofilms by regulating the quorum sensing communication system (K. Golberg et al.,2013). These bacteria play an important role in regulation of bio-fouling by secreting antimicrobial compounds and engage in nitrogen fixation, nitrogen cycling, sulfur cycling and physiology maintenance of the corals (Lesser et al. 2004; Shnit-Orland & Kushmaro 2009; Medina 2011). The most common Bacterial phyla found are Proteobacteria (class *Gammaproteobacteria, Alphaproteobacteria), bacteroidetes, Cyanoobacteria Firmicutes* and *Tenericutes.* Archaeal phyla comprise of *Thaumarchaeota and Euryarchaeota*. Dominant culturable bacteria are *Vibrio, Psuedoalteromonas, Photobacterium, Bacillus, Shewanella* and *Ruegeria*. Metagenomic analysis studies shows wide varieties of species such as *Vibrio, Endozoicomonas, Ruegeria, Paenibacillus, Mycoplasma, Psuedoalteromonas, Fusibacter, Marinifilum, Bacillus, Candidatus* and undescribed groups associated with Caldilineaceae, Flavobacteraceae and Alteromonadaceae (Huggett and Apprill, 2019).

CHAPTER II

LITERATURE REVIEW

LITERATURE REVIEW

Understanding the coral associated holobionts architecture and their major stress response pathways helps in conservation of the corals. This laying foundation, reported that *Montipora capitata* foreign gene acquisition via a bacterial gene transfer agent and major stress response are used to predict regulatory components of the transcriptional networks. The Hawaiian reef restoration and conservation has studied the adaptive potential of Reef building corals in both natural and human influenced environments facilitatating functional and population genomic studies on reef associated microbes. *M.capitata* is an ideal species that is broadcast spawning coral, dominant reef builder in lagoon and fringing reef sites and endemic to the Northwest and main Hawaiian islands. It is found that *M.capitata* genome assembly is nearly double the size of other sequenced corals eg. *Acropora digitifera* and *Stylophora pisitillata*. Using single copy orthologous genes of 10 corals and 2 sea anemone species a phylogenic tree was built and identified for horizontal gene transfer of a bacterium-derived 4-gene cluster in genome contig (K. Golberg et al., 2103). HGT(Horizontal Gene Transfer) candidates are flanked on both sides by sequences of eukaryotic(metazoan) origin and the bacterial genes are putatively of proteobacterial provenance (Arup panda., et al 2018). Reports also state that HGT candidates genes are expressed under the different temperatures and pCO2 and the evolutionary history shows the adaptive traits such as protection from UVR (Ultra Violet Radiation) and stress from reactive species, many of which are lineage-specific(K. Golberg et al., 2103).

The microbiome of the corals is dynamic and challenging to predict. It is extensively influenced by environmental factors and microbe-microbe interactions. It is important to study the microbial community structure of the coral as the coral reef health is deeply depend on it. The dynamic model of *Psuedodiploria strigosa,* the symmetrical brain coral of Mussidae family was established by keeping the Temperature as extrinsic factor of microbiome in the Surface Mucus Layer (SML) and microbial network as an intrinsic factor (Lima et al., 2020). Bacterial colonies isolated from *Favia sp.* are found to have antifouling properties to attenuate the formation of

biofilm of indicator strians of *Psuedomonas aeruginosa* and *Acinetobacter baumannii* (K. Golberg et al., 2103).

Montastrea cavernosa orange morphs with aposematic coloration recorded with the significant increase in the orange carotenoid protein of cyanobacterial community which is quantified by 16SrRNA reads and flow cytometry. The orange carotenoid protein is distinctive to cyanobacteria, which has two main roles as photoreceptor in the blue region of the spectrum and in photoprotection of photosystem II from high irradiance by triggering non-photochemical quenching in the phycobilisome. The microbiome of orange color morphs shows the ability to fix nitrogen by expressing more nitrogenase (nifH) transcripts, which helps in the holobiont on oligotrophic waters; transcriptome that expresses genes related to immune response and apoptosis which helps is maintaining and regulating the unique symbiont population. (Jessica K.jrett et al., 2017).

The genomic studies on *Pocillopora damicornis* (one of the abundant and widespread coral) and other publicly available coral genomes scelractinian corals have a core genome enriched in basic housekeeping functions such as cellular signaling and stress response pathways indicating diversified immune system at multiple taxonomic levels when compared to genomes of other anthozoan groups (Actiniaria, Corallimorpharia) and two basal metazoan out-group phyla (Porifera, Ctenophora) (R.Cunning et al.,2018).

Porcillopora damicornis is DAPI-stained and observed under the High-speed Laser scanning con-focal microscope and viewed into a poly to observe a bacterium shedding mechanism. It is found that corals shed bacteria as a potential mechanism by which coral associated bacterial abundances are regulated under organic matter stress (Melissa Garren and Farooq Azam ., 2011).

Table 2.1 Coral associated bacteria from diverse coral species and their stress response activities

CORAL	BACTERIAL COLONIES ASSOCIATED	STUDIES	REFERENCES
Psuedodiploria strigosa (Mussidae)	30 bacterial colonies isolated *Alphaproteobacteria Vibrionales, Flavobacteriales, Rhodobacterales, Alteromonadales, Vibrio sp Bacilli, Gammaproteobacteria*	Modeling of the coral microbiome with the temperature as a extrinsic factor and microbial network as the intrinsic factor	Lima et al., 2020
Platygyra sp., Porites sp., Fungia granulosa, Favia sp., Stylophora sp., Acanthastrea sp., Pocillopora sp. Acanthastrea sp	120 bacterial colonies isolated	Isolation of Biofouling compound which act by inhibiting quorum sensing of indicator strains and AHL (Acyl homoserine lactone) induction activity found positive in 30 % of the bacterial isolates	K. Golberg et al., 2011; K. Golberg et al., 2013

Mussismilia hispida, Madracis decactis	*Proteobacteria Pseudomonadales sp*	SEED and Pfam functional profiles of metagenomes of two Brazilian coral species - shows high profiles of Transport proteins involved in bacterial colinization of corals	Carlos.C et al., 2014
Millepora, Poritidae, Pocilloporidae, Acroporidae, Milleporidae, Euphylliidae, Mussidae	1637 unique bacterial in 16S sequencing: (*Alphaproteobacteria*) *Kordiimonadales;* (*Gammaproteobacteria*) *Spirochaetaceae* , (*Oceanospirillales*) *Endozoicomonas,* Ectothiorhodospiraceae, *Vibrionaceae, Congregibacter, (Alteromonadales) Shewanellaceae, Pseudoalteromonadaceae, Clostridiaceae, Desulfobacteraceae, Bacteroidales*	Characterization of coral associated bacterial colonies that challenges the coral health using Nanopore sequencing	Quentin Carradec et al.,2010
Acropora millepora	*Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria*	Total holobiont protein is dropped to approximately 44% in the Bleached coral, demonstrated during the warm sea water temperature periods and DGGE analysis shows change in microbial	David bourne et al.,2008

		community.	
Montastrea annularis	67 bacterial isolates *Alpha-proteobacteria*-20% *Gamma-proteobacteria*-72% *Vibrionales* *Alteromonadales*	Production of inhibitory compunds at 25°C and 31°C against the 2 model coral pathogens *Vibrio shiloi* and *Vibrio coralliilyticus*	Krystal L. Rypien etal.,2010

CHAPTER III

AIM AND OBJECTIVES

AIM

The aim of this project is to carry out Studies on Bacterial Communities associated with Corals.

OBJECTVES

1. Sampling of Corals from Gulf of Kutch in collaboration with the Zoological Survey of India.
2. To isolate the culturable bacteria from the coral tissues isolated.
3. To isolate metagenomic and isolated bacterial DNA from the sampled coral tissues.

CHAPTER IV

MATERIALS AND METHODOLOGY

METHODOLOGY:

3.1 Sample collection

Live corals (5cm length) are collected from the Narara Marine National Park located in Gulf of Kutch coast at Lat 22.467377° Lon 69.678189°on 16th October, 2022. Triplicate samples of same species from the Wild site and coral trans-located site of ZSI-IOCL project are collected via SCUBA diving at a depth of 5 to 8 m. The samples are collected in a sterile plastic containers along with the seawater. After reaching the port samples are rinsed with sterile distilled water. Samples are then shifted to containers having sterile artificial seawater and transported in the ice boxes by maintaining at 4°C during the time of transportation to the laboratory.

Parameters such as Temperature, Pressure, salinity, Chlorophyll -a (Fluorescence), Total dissolved solids, Oxygen Partial Pressure and Barometric pressure were measured.

3.2 Isolation of Culturable bacteria from coral tissues

Tissues of 6 *Goniopora* coral species were inoculated in Zobell Marine Broth of different strength (100%, 50%, 25%, 10%) and Artificial Sea Water. The inoculated broths were incubated at 30°C for 24h in shaking conditions. After sufficient growth was observed, 500 µL of the broth is collected in sterile 2 mL centrifuge tubes. Serial dilutions were prepared and spread plated on different strength media of Zobell Marine Agar and Artificial Seawater plates containing agar. The plates were incubated at 30°C The plates were daily observed for growth for a period of 1 week. 48 hrs & 1 week. Bacterial colonies were selected and isolated by quadrant streak method.

Bacterial colony characteristics are observed for their morphological characteristics. Pure isolates obtained were preserved in the 50 % Glycerol stock.

3.3 Gram Staining

To study the Gram nature of the isolated bacterial colonies, Gram staining was done by using "HIMEDIA, Gram stain, K001" kit . The results were observed under the Microscope (10X,40X and 100X oil immersion).

3.4 Isolation of Genomic DNA

1.5-2 mL of overnight grown culture was taken and genomic DNA was isolated using HiPurA® Bacterial Genomic DNA Purification Kit by following the instructions mentioned in the manual provided. The concentration of the isolated DNA was checked using Qubit 2.0.

3.5 Metagenomic DNA Extraction

Coral samples were homogenized with the help of Liquid Nitrogen using a sterile mortar and pestle. Macerated coral samples were then collected in the sterile 50 ml centrifuge tubes and stored at -20°C. DNA from the macerated coral samples were extracted using Himedia HiPurA Metagenomic Soil DNA Kit by following the instructions in the manual provided. The concentration of the isolated DNA was checked using Qubit 2.0

3.6 Precipitation of Metagenomic DNA with Sodium Acetate and Ethanol

Metagenomic DNA of homogenized corals was extracted four times, due to the low concentration of the DNA. The purified DNA was pooled together. 2 µL of 3M Sodium Acetate (pH:5.5) and 1 mL of 100% Ethanol were added to the pooled DNA . The sample was incubated overnight at -20°C and centrifuged for 30 minutes at 4°C at10000 rpm. After decanting the supernatant the transparent DNA pellet is washed with 1mL 70% Ethanol. After air drying the DNA pellet is re-suspended in 10 µL TE buffer.

3.7 Gel Electrophoresis

Agarose gel electrophoresis was performed to check the genomic DNA bands. 0.7% agarose gel was prepared by dissolving 0.28 g of agarose in a 40 ml 1x Tris-acetate-EDTA (TAE) buffer. 5 µL of extracted genomic DNA was electrophoresed. Electrophoresis was performed for 1.5 hours at room temperature with a constant voltage of 100 V.

CHAPTER V

RESULTS AND DISCUSSIONS

RESULTS AND DISCUSSIONS

4.1 Sampling and physio-chemical data collection

The physico-chemical parameters of the sea water were checked at the sampling site using Aqua TROLL 500 probe Multi-parameter Sonde. Reading shows that the sampling site has extreme conditions, where the corals are well adapted to survive.

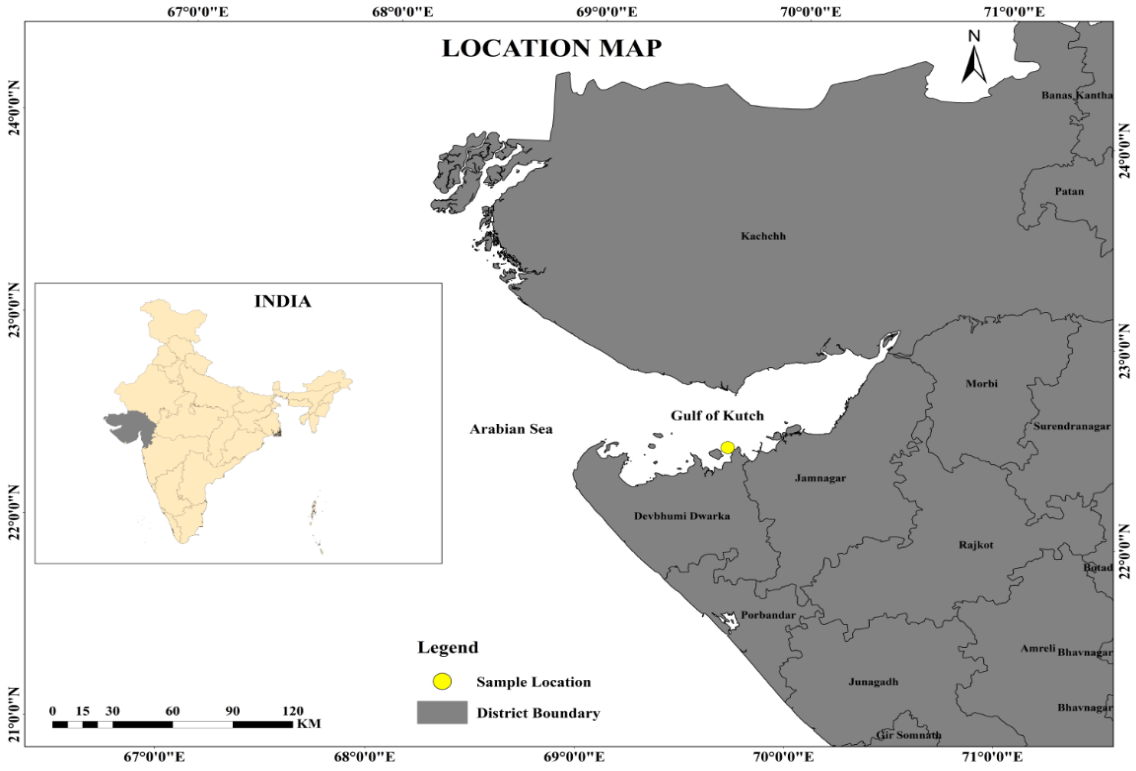


Fig 4.1 Map of sampling location generated using ArcGis10.2 software: the Narara Marine National park located in Gulf of Kutch coast at Lat 22.467377° Lon 69.678189°

Table 4.1: Surface water parameters of the study area at the time of sampling

PARAMETERS	FIELD READINGS	OPTIMUM SCALE
pH	9.3	7.8-8.4
TEMPERATURE	41°C	23-29°C
SALINITY(psu)	34	32-42
OXYGEN REDUCTION POTENCIAL (ORP)mv	206	300-400(Ocean)
TOTAL DISSOLVED SOLIDS (ppt)	33	30-40
CHLOROPHYLL-a FLUORESENCE(RFU) ($mg/m^{3)}$	0.8	0.2-0.6
OXYGEN PARTIAL PRESSURE (PaO2)	189.5918	-
CONDUCTIVITY (S/m)	52388.21	-

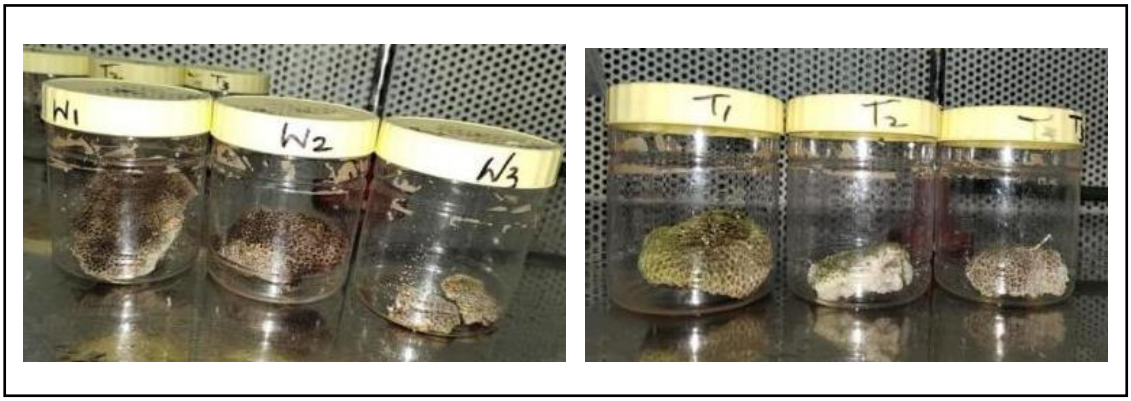


Fig4.2 Coral samples collected from the study site

4..2 Isolated bacterial colonies

Triplets of *Goniopora* sp samples are collected, at ZSI-IOCL coral translocated site and wild site Gulf of Kutch coast. Three (W1,W2,W3) from the Wild and three (T1,T2,T3) from the translocated coral colonies were processed sequentially. 20 bacterial colonies were isolated.

Table 4.2: Morphological characteristics of 20 Bacterial isolates from GOK Goniopora sp in different strength media

SI: NO	PLATE	SIZE	COLOUR	TRANSPARENCY	FORM	ELEVATION	MARGIN	TEXTURE
	ZMA PLATES							
	100%							
1	GKWC1	>1mm	White	Opaque	Irregular	Convex	Undulate	Smooth
2	GKWC2	>1mm	White	Translucent	Punctiform (concentric)	Flat	Lobate	Rough
3	GKWC3	<1mm	Cream white	Opaque	Circular	Raised	Entire	Smooth
4	GKWC4	>1mm	Light Brown	Opaque	Circular	Convex	Entire	Glistening
	50%							
5	GKTC5	>1mm	Light Brown	Opaque	Circular	Convex	Entire	Smooth
6	GKTC6	<1mm	Yellow	Opaque	Punctiform	Convex	Entire	Smooth
7	GKTC7	>1mm	White	Opaque	Circular	Pulvinate	Entire	Smooth
8	GKWC8	>1mm	Light Brown	Translucent	Irregular	Raised	Lobate	Smooth
	25%							
9	GKTC9	>1mm	Pink	Opaque	Irregular	Pulvinate	Undulate	Smooth
10	GKTC10	<1mm	mild yellow	Opaque	Punctiform	Raised	Entire	Glistening
11	GKTC11	>1mm	White	Opaque	circular (concentric)	Raised	Curled	Rough
12	GKTC12	>1mm	Cream white	Translucent	Irregular	Raised	Lobate (spreading)	Rough
13	GKTC13	1mm	Cream white	Translucent	Irregular	Flat	Curled	Smooth
	10%							
14	GKWC14	<1mm	Cream white	Translucent	Punctiform	Flat	Curled (concentric)	Rough
15	GKWC15	<1mm	Cream white	Translucent	Punctiform	Flat	Curled (concentric)	Smooth
16	GKWC16	>1mm	Cream white	Opaque	Irregular	Raised	Lobate	Smooth
	ASW+AGAR							
17	GKTC17	>1mm	White	Translucent	Irregular	Raised	Lobate(concentric)	Smooth
18	GKTC18	<1mm	White	Translucent	Irregular	Raised	Lobate(concentric)	Smooth
19	GKTC19	<1mm	White	Translucent	Punctiform	Raised	Undulate	Smooth
20	GKTC20	<1mm	White	Translucent	Punctiform	Raised	Undulate	Smooth

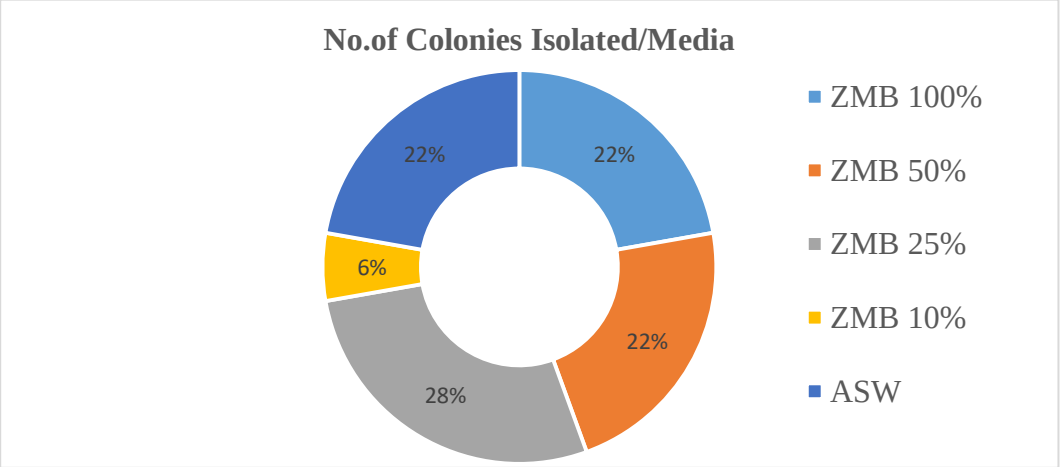


Fig 4.3 Percentage of isolated colonies in different diluent media

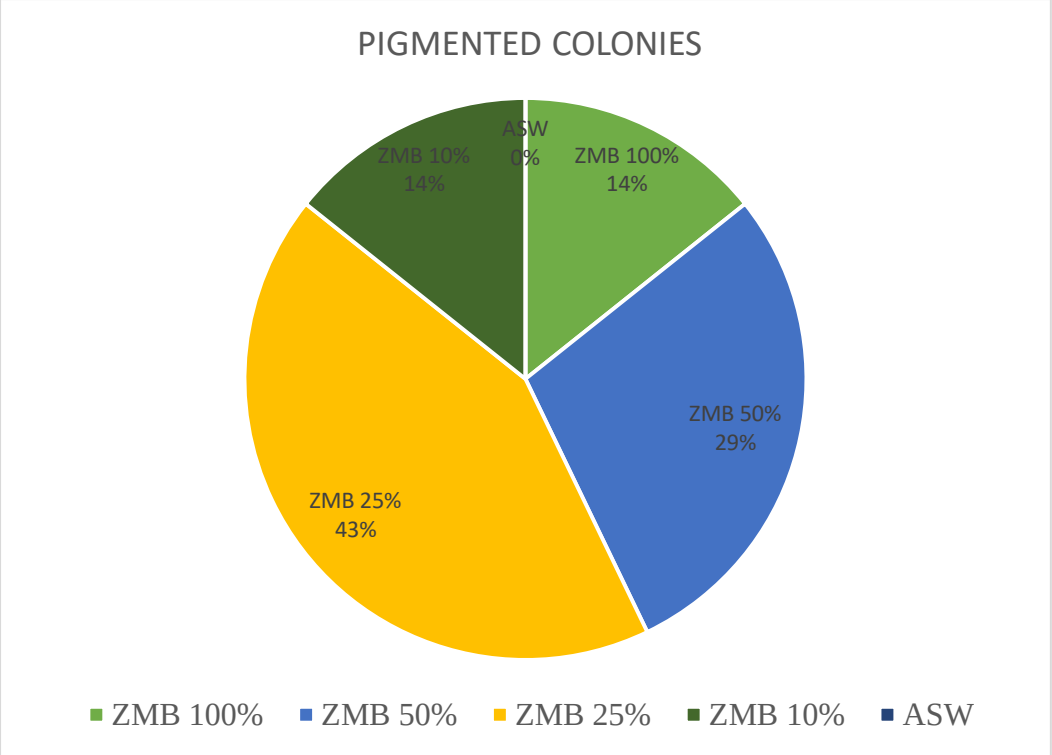


Fig 4.4 Graph showing the percentage of pigmented colonies in different strength media

Table 4.3: Pure culture of the bacterial isolates from *goniopora sp*

BACTERIAL COLONY PICTURE	
 GKTC5	 GKTC9
 GKTC6	 GKTC10

Table 4.4: Gram nature and shape of the Bacterial isolates from *Goniopora sp*

SAMPLE	ISOLATES	GRAM NATURE	SHAPE
	ZMB 100%		
	GKWC1	Positive	Cocci
	GKWC2	Negative	Rod
	GKWC3	Positive	Rod
W1	GKWC4	Negative	Rod
	ZMB 50%		
	GKTC5	Positive	Rod
	GKTC6	Positive	Cocci
T3	GKTC7	Positive	Rod
W2	GKWC8	Negative	Rod
	ZMB 25%		
	GKTC9	Negative	Rod
T3	GKTC10	Negative	Rod
	GKTC11	Positive	Rod
	GKTC12	Negative	Rod
T1	GKTC13	Negative	Rod
	ZMB 10%		
W1	GKWC14	Positive	Rod
	ASW		
	GKTC17	Negative	Rod
T1	GKTC18	Positive	Rod
	GKTC19	Negative	Rod
T2	GKTC20	Positive	Rod

Table 4.5: Gram strain pictures of isolated colonies from *Goniopora sp*

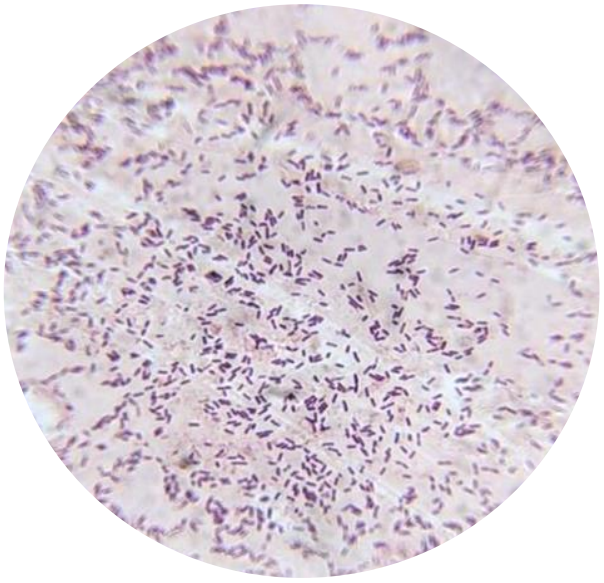
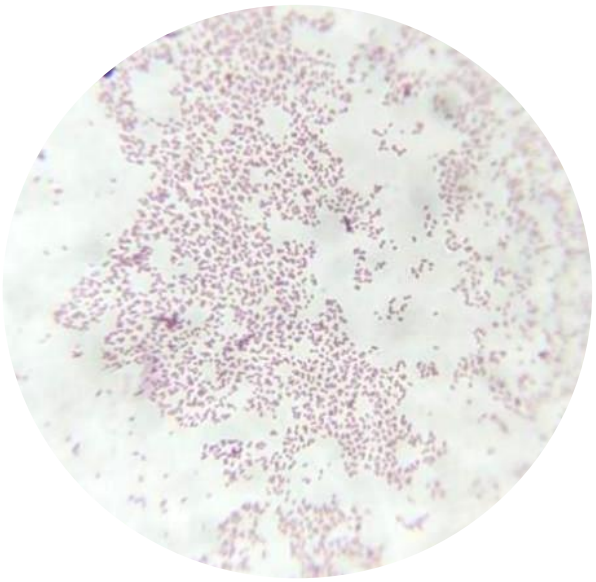
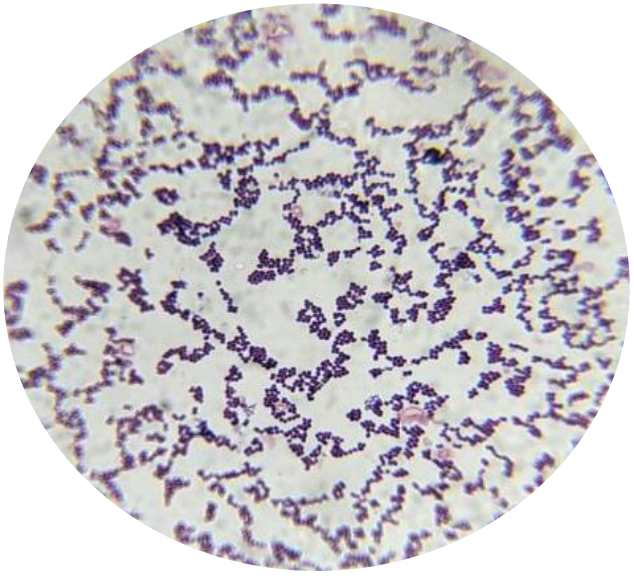
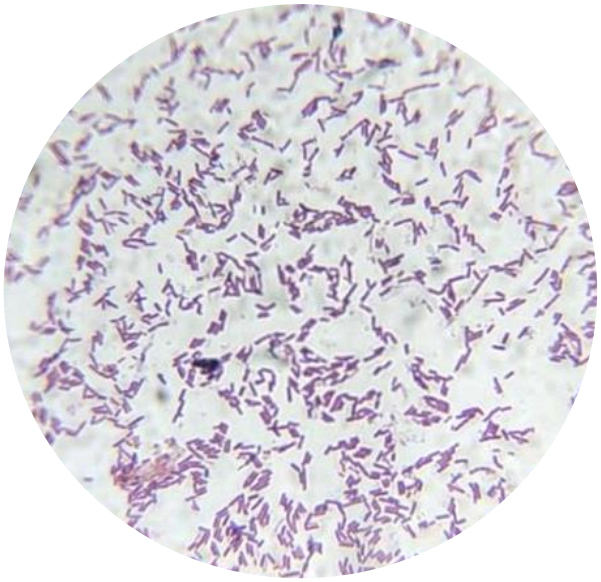
GRAM STAIN	
 GKTC18	 GKTC9
 GKTC6	 GKTC10

Fig 4.5 Percentage of Gram Positive and Gram Negative stains in different diluent media

4.3 Metagenomic DNA Quantification:

DNA extraction was carried out for 6 coral macerated samples. As the concentration of the DNA extracted from each samples were low. The samples were extracted four times and pooled together. The DNA was then precipitated followed by quantification.

Table 4.5 : DNA Quantification table for Metagenomic DNA

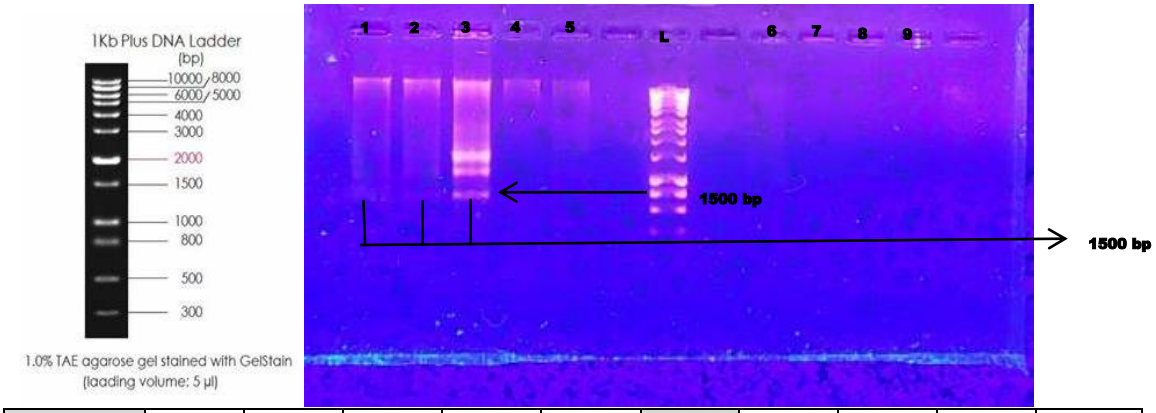
SAMPLE	DNA PRECIPITATED (µg/mL)
W1	3.86
W2	8.72
W3	22.5
T1	3.48
T2	4.12
T3	2.86

4.4 DNA Quantification of isolated bacterial colonies

9 Bacterial samples were selected for DNA isolation. 5 Bacterial samples were isolated with thick bands of size around 1500 bp and 4 bacterial colonies with fades bands.

Table 4.6 : DNA Quantification table for the isolated bacterial DNA samples

SAMPLE	DNA CONCENTRATION(µg/mL)
GKWC8	**13.4**
GKWC14	**8.28**
GKTC9	**30.4**
GKTC12	**72.4**
GKTC13	**11.7**
GKTC10	**16.4**
GKTC5	**5.36**
GKTC6	**2.86**
GKTC11	**2.93**



LANE NO	1	2	3	4	5	L	6	7	8	9
SAMPLE	GKWC8	GKTC9	GKTC12	GKTC13	GKTC10	1Kb DNA ladder	GKTC5	GKTC6	GKTC11	GKWC14

Fig 4.6Gel Electrophoresis of the isolated bacterial DNA samples

DISCUSSIONS

In total 8 bacterial colonies were successfully isolated from wild samples and 12 bacterial colonies were isolated from the translocated samples. The bacteria isolated from the translocated coral tissues has different morphological characters when compared to those isolated from the original site . It may be considered that the same species of coral fragments,when shifted to translocated site has different bacterial diversity.

In comparison to the highly concentrated ZMB 100% and ZMB 50% medium more isolated bacterial colonies were isolated from the 25% strength ZMB medium. than. This indicates that bacteria can grow in nutrient depleted environment than the nutrient-rich environment. As the bacterial colonies are isolated from coral tissue, which is habitat in the oligotrophic zone (Ion joint., et al.,2010).

As the physicochemical parameters report on surface reef water shows an optimum increase in the level of alkalinity (pH 9.3) it provides a healthy environment for bacteria associated with corals to actively involve in active biogeochemical cycles such as carbon, sulphur and nitrogen cycle are involved in detoxifying the toxins in the host, controlling nutrient metabolism, food webs and organism life cycle (Ainsworth et al., 2010), The nitrogen fixing bacteria such as *Cyanobacteria Synechococcus, Mastigocoelus , Pleurocapsa* (Charpy et al., 2012), *Brevundimonas, Chroococcidiopsis* (Brocke et al., 2018; Cai et al., 2018), are known to increases potential of the chemo-symbiotic endosymbionts *Candiatus thiodiazotropha and candiatus thoiobios* to utilize the nitrogen source and nurture the photosynthetic activity of the cyanobacteria (responsible for the carbonate cycle and their ability to incorporate carbonate material in corals)(Arp et al., 1999; Charpy et al., 2012).

The optimum increases in the level of alkalinity (pH9.3) also shows the profusion in chlorophyll a fluorescence level (0.8 (RFU) ($mg/m^{3)}$ in the coral reef zone, which indicates the presence of healthy photosynthetic organism (Pedro R.Frade.,et al,2020).

The abundance of gram negative stains of culturable bacterial is in accordance to reported from the results of research papers (Lima et al., 2020;K. Golberg et al., 2011;K. Golberg et al., 2013;Krystal L. Rypien etal.,2010;Quentin Carradec et al.,2010;Lima et al., 2020),that demonstrate shows the presence of more Gram negative bacterial families such as *Rhodobacteraceae* and *Proteobacterial* that were isolated from the shallow inshore reef coral tissue. The metagenomic analysis of bacterial communities associated with *Goniopora sp* collected from GOK Narara Marine National Park, shows 60 % abundance in *Proteobacteria* phylum and relative abundance in *Cyanobacteria* (Zarna.Z. Patel.,et al , 2023).

Presence of pigmented colonies are observed more in 25% diluted ZMB and 50% diluted ZMB. Pigmented bacteria isolated from low strength media has potential antagonistic activity against marine bacterium (VS Jayasree ., et al, 2020)

SUMMARY

SUMMARY

- Triplet samples of corals are collected from the Narara Marine National Park located in Gulf of Kutch coast at Lat 22.467377° Lon 69.678189.
- Coral samples are homogenized and Metagenomic DNA is isolated.
- Morphologically distinct 20 bacterial colonies are isolated and genomic DNA is also isolated to identify the culturable bacteria associated with coral samples.
- Presence of More gram negative bacterial colonies may indicate potential coral associated bacterial colonies that are dominant in the shallow waters and oligotrophic water.
- Pigmented colonies are isolated on the lower strength ZMA plate.

FUTURE PROSPECTS

- Analysis of the isolated Metagenomic DNA.
- Analysis of genomic DNA data of the isolated bacterial colonies

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APPENDIX

ZOBELL MARINE AGAR MEDIA

Ingredients Gms / Litre

- Peptic digest of animal tissue 5.000
- Yeast extract 1.000
- Ferric citrate 0.100
- Sodium chloride 19.450
- Magnesium chloride 8.800
- Sodium sulphate 3.240
- Calcium chloride 1.800
- Potassium chloride 0.550
- Sodium bicarbonate 0.160
- Potassium bromide 0.080
- Strontium chloride 0.034
- Boric acid 0.022
- Sodium silicate 0.004
- Sodium fluorate 0.0024
- Ammonium nitrate 0.0016
- Disodium phosphate 0.008
- Agar 15.000
- Final pH (at 25°C) 7.6±0.2

ARTIFICIAL SEAWATER MEDIA

Ingredients Gms / Litre

- Sodium chloride (NaCl) -17.52
- Magnesium sulphate (MgSO4) - 12.32
- Calcium chloride dihydrate (CaCl2.2H2O) - 0.14
- Potassium chloride (KCl) - 0.74
- Diammonium phosphate ($(NH_4)_2HPO_4$) -0.13
- Tris Base NH2C(CH2OH)3 - 6.05
- pH - 7

50X TAE buffer

- 242 g tris base in ddH2O 57.1mL glacial acetic acid
- 100mL 0.5M EDTA solution (pH 8.0)
- Adjust volume to 1L.

1X TAE buffer

- Add 10mL 50X TAE buffer in 490mL ddH2O

Ouriginal

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STUDIES ON BACTERIAL COMMUNITIES ASSOCIATED WITH CORALS
Dissertation
thesis submitted to Goa University
In partial fulfillment of the requirement for the degree of
Master of Science in Marine
Biotechnology
for the academic year 2022-23 By
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