ISOLATION AND IDENTIFICATION OF BACTERIA FROM GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii (De man ,1879)*

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Abstract: The Giantfreshwater prawn *Macrobrachium rosenbergii* commonly called as scampi , farmed *crustacean* , species . we used in the study aim to identify the type of bacteria and stain present in gut of Giant freshwater prawn *M.rosenbergii* . A sample were collected from the Mallana Sagar Reservoir the Latitude 17.964° and Longitude 78.7472°are approximately located in Siddipet , dist. of Telangana state . This sample should be identified the selective species and also observed morphological, physical characteristics of selective species and then dissected and extracted the Gut sample from Giant freshwater prawn *M.rosenbergii* . Prepared Nutrient Agar media and sample taken to serial dilution and incubated the Pure culture media . after 24 hours in next day . we observed the bacterial about 30 colonies in sample 10^{th} , then again prepared Nutrient Agar and incubated sub- culture , after 24 hours next day prepared bacteria smear, on slide and observed bacteria stain. This abstract examines Gram - positive bacteria were retained the crystal violet and appeared Purple and Blue colour , Gram - negative bacteria were not retain crystal violet but are count stained with safranin appeared Pink and red colour , both bacteria can exhibit various shapes *cocci*- round ,*bacilli* – rod , *vibrio* – comma,*coccobacilli* – short oval rods etc. The range of Gram – positive and Gram – negative bacteria observed and identified among the isolates 60% were Gram – positive bacteria and 40% were Gram – negative bacteria **.** Rod shaped bacteria *Bacilli,Escherichia coli, Bacillus , lactobacillus ssp , salmonella ssp,pseudomonas aeruginosa* and comma shaped bacteria *vibrio,* Round or spherical shaped bacteria *cocci , Staphylococcus, aureus ,streptococcus , enterococcus, micrococcus* etc , these types of bacteria associated in gut of Giant freshwater prawn *Macrobrachium rosenbergii.*

Keywords: Malla Sagar Reservoir , Siddipet,Telangana ,Latitude , Longitude , Giant freshwater prawn , Bacteria , Sample , Gut , Nutrient Agar Media , Pure Culture , Sub Culture , Gram Staining.

INTRODUCTION

Giant freshwater prawn *Macrobrachiumrosenbergii* belongs to Phylum *Arthropoda*, Class *Malacostraca*, Order *Decapoda*, Family *Palaemonidae*, Subphylum *Crustacea*, genus *Macrobrachium*, Species *Macrobrachiumrosenbergii* . Historical Classification and Discoed by The Dutch carcinologist Johann Dietrich Eduard De Man first described *Macrobrachium rosenbergii* in 1879. Specimens from Southeast Asia, particularly Malaysia and India, were used for the first description. Placement of the Original Genus Like many freshwater prawns, it was first assigned to the genus *Palaemon*. Large freshwater prawns with lengthy second chelipeds (claws) were later incorporated into the genus *Macrobrachium*. The species *"rosenbergii"* was named after the Dutch researcher and explorer Willem Frederik Robert Rosenberg. Evolution of Classification in the 19th Century (Late 1800s) *Crustaceans* were broadly classified based on external morphology. De Man's research contributed to the classification of *Macrobrachium* as a separate genus on the basis of variations in chelae (claws) and rostrum anatomy. 20th Century With expanding knowledge in anatomy, the development of eggs, and larval development, the classification of freshwater prawns became more further developed. *Macrobrachium rosenbergii* position in the genus was strengthened even more by the significant research conducted on its aquaculture potential. Late 20th to Early 21st Century: Molecular phylogenetics (DNA analysis) confirmed the monophyly of the genus *Macrobrachium*, and placed *M. rosenbergii* within the *Palaemonidae* family with strong support. Its strong evolutionary link to other Southeast Asian Macrobrachium species was established by mitochondrial DNA and rRNA research. Current Taxonomic Status of Today, *Macrobrachium rosenbergii* is recognized as the biggest species of freshwater prawn in the world. Economically important in aquaculture worldwide, especially in Asian countries, the Pacific, and Latin America. This species' life cycle is complicated; it includes freshwater adulthood and brackish water larval stages.

As a Giant freshwater prawn , *Macrobrachiumrosenbergii* (De man, 1879) commonly called as Scampi , is a very important aquaculture species valued as nutritious food source ,that are the one of most economically important crustaceans cultured in aquaculture systems especially in south Asia and India an extensive amount of in tropical and subtropical areas .it is a mainstay of inland fisheries and aquaculture that is focused on exporting in India and other countries because of it grows quickly , can be cultivated in many different types of farming , and is valuable lot of wealth but as prawn farming has become more intensive , they have become more highly susceptible to microbial infections , especially in hatcheries and aquaculture species Fresh water prawn is a commercially valuable species due to its food source and growth potential. The growing demand for scampi in domestic and export markets leads to the development of large-scale, high-stocking-density systems with intensive feeding practices. This economically important plant is now being grown in rice fields, orchard gardens, and riverbank pens. Therefore, disease is unavoidable in uncontrollable cultural models.(M. Prakesh and N.karmagan 2013).Antibiotics are also increasingly being used to manage bacterial populations and preserve an environment that's healthy for prawn production. A number of antibiotics are used at present to treat diseases caused by bacteria as well as control the bacterial population in prawn farms and hatcheries. The

bacteria that are antibiotic is one of the possible side effects of using antibiotics for therapy. Resistance was a condition that spread to pathogenic bacteria and decreased the effectiveness of antibiotic treatment for diseases caused on by the resistant pathogens (Frappaolo et al., 1986). Luminescent bacterial disease due to Vibrio spp. (Tonguthai, 1995) has been reported to cause serious mortalities in *M.rosenbergii* hatcheries. Lavilla-Pitogo et al., (1990) and (Karunasagar et al., 1994) reported luminescent vibriosis to be a major problem causing significant mortalities in systems employing saline waters such as *Penaeus monodon* hatcheries. The larval rearing practices favor the rapid multiplication of bacteria in the system. However, most bacteria are part of the commensal flora and only Some of them may be opportunistic pathogens.In culture systems, factors like water source and quality, diet, stocking density, and habitat structure differ from the natural environment, potentially resulting in a different gut microflora (Prieur et al., 1990; Strom) In shrimp aquaculture, vibriosis is a serious disease issue that leads to high mortality rates and significant financial losses in everywhere (Brock and Lea Master, 1992; Lightner, 1988; Mohney et al., 1994).(Shotts and Gratzek 1977) were among the pioneers who highlighted bacterial infections in fish and prawns, emphasizing the role of aquatic bacteria in disease outbreaks. Since then, considerable attention has been given to bacterial flora associated with freshwater prawns, especially with the growth of the aquaculture sector . According to (Moriarty 1999), early research has demonstrated that altering the bacterial species makeup of the rearing water and the prawns' digestive tracts can boost prawn productivity. Nevertheless, the Originating bacterial makeup of the freshwater prawn and its culture medium has not yet been completely determined. As stated by (Lalitha and Surendran 2004), the total quantity and variety of bacterial species in freshwater prawns produced in relation to their culture environment have been considered to be useful indicators to define the quality of harvested prawns and the success of production. Furthermore, particular data on the species composition and number of bacteria in *M. rosenbergii* and its rearing settings that appear to be in good health are required. Furthermore, having this information helps prawn growers predict probable disease epizooticsand concomitant opportunity to instigate management action to prevent these outbreaks. Pertinent information on the bacterial microbiota of the hatchery-produced freshwater prawn in the Philippines is currently not available. Thus, the present study was undertaken to investigate quantitatively and qualitatively the bacteria present in the rearing water, eggs and larvae of *M. rosenbergii*. In shrimp aquaculture, vibriosis is a serious disease issue that leads to high mortality rates and significant financial losses in everywhere (Brock and Lea Master, 1992; Lightner, 1988; Mohney et al., 1994). It is most commonly thought of as pathology in shrimp, however reports of a primary sickness brought on by extremely virulent strains of Vibrio sp. have also been made (De la Pena et al., 1993). *Vibrio harleyi*, *V. alginolyticus*, *V. parahaemolyticus*, and *V. anguillarum* are the main genera responsible for vibriosis in shrimp (Lightner, 1988; Lightner, 1996; Jiravanichpaisal et al., 1994). These diseases affect all stages of raising, from hatching tanks to grow-out ponds. But since both species and stage-level reports of specificity have been made, strains that cause larval and juvenile vibriosis are thought to be distinct, even when belonging to same species. The probiotic organisms are considered as an preference to antibiotics in culture practice to enhance immunity to the shrimps in monoculture against various pathogenic bacteria (Ajitha et al., 2004)Generally, probiotic bacteria don't synthesize remainders

or specificresistance in submarine organisms, probiotics as a alternate for chemical precious antibiotics have come a recent disquisition content of disquisition in monoculture (Akhter et al., 2015)

The study by (Martin W Hahn, at al 2019)discusses the importance of preventing the spread and growth of microorganisms in order to conduct comprehensive research on their biological environment and function. The availability of cultures facilitates high-quality genome research, Eco physiological tests, and the description of new prokaryotic species. However, much of the microbial variety has not been cultivated, and access to microorganisms through cultivation techniques remains limited. The chapter explores developments in prokaryotic cultivation techniques and potential causes. This research explores the evolution of gut microbial communities in the molting stages of the Giant freshwater prawn *Macrobrachium rosenbergii*. The study reveals that crustaceans destroy a significant portion of their gut and microbiome with every molt, affecting the microbial communities that could influence molt cycle characteristics. The study used 454 pyrosequencing to examine the diversity of the 16S rRNA gene (Elenimente at al 2016).The method of estimation for investigations using repeated dilution is described by (Charles at al. 2014) in the Journal of Microbiological Methods. This method involves measuring bacteria in ambient or pathogenic samples and estimating microbial counts using the serial dilution technique. Duplicate plates are not needed to estimate the number of living bacterial organisms from a single diluting plate. The differential staining method, which at first was created in 1884 by Danish physician Hans Christian Gram, remains the primary method of taxonomic classification and bacterial identification. During a multi-step, sequential staining procedure, bacteria are divided into four groups: Gram-positive *cocci*, Gram-negative *cocci*, Gram-positive rods, and Gram-negative rods, depending on their cell shape and cell wall structure. The Gram stain is advantageous for determining whether tissue culture materials are contaminated by bacteria or for studying the morphological characteristics and Gram stain characteristics of bacteria isolated from isolated or mixed bacterial cultures.

METHODOLOGY

1. Collection of Sample :-

A sample of giant fresh water prawn *(Macrobrachium rosenbergii)*were collected on the day of experiment from the Mallana SagarReservoir the latitude 17.964° and longitude 78.7472° are approximately located in Siddipet ,dist. of Telangana state . which collected sample were brought for experiment to isolate and identify the bacteria stain in gut from Giant freshwater prawn *(M.rosenbergii)*In theCRL (Central Research Laboratory) Of Government Degree and PG College (Autonomous) Siddipet .

Identification of selective species *Macrobrachium rosenbergii (DeMan ,1879)*

Giant freshwater prawn *M. rosenbergii*

Taxonomic classification:-

CLASSIFICATION	
KINGDOM	ANIMALIA
PHYLUM	ARTHROPODA
SUB PHYLUM	CRUSTACEA
CLASS	MALACOSTRACA
ORDER	DECAPODA
FAMILY	PLAEMONIDAE
GENUS	*MACROBRACHIUM*
SPECIES	*M. rosenbergii*

Fig:- 1,*Macrobrachiumrosebergii*

IUCN STATUS :-Giant Freshwater Prawn the IUCN Red List of Threatened Species most recently evaluated *Macrobrachium rosenbergii* in 27 march 2012 The species *Macrobrachium rosenbergii* is classified as Least Concern. And fully recovered in 31 October 2021

External Anatomy of Giant freshwater prawn (*Macrobrachium rosenbergii)*

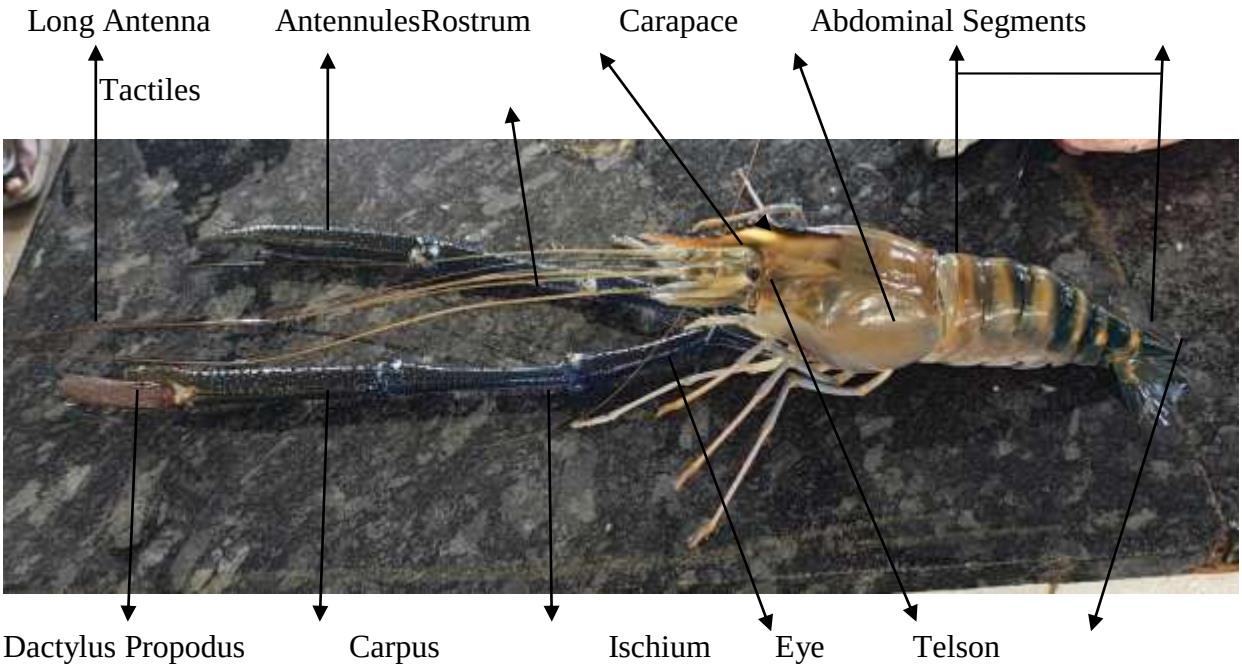

Fig :- 2 *M.rosenbergii*

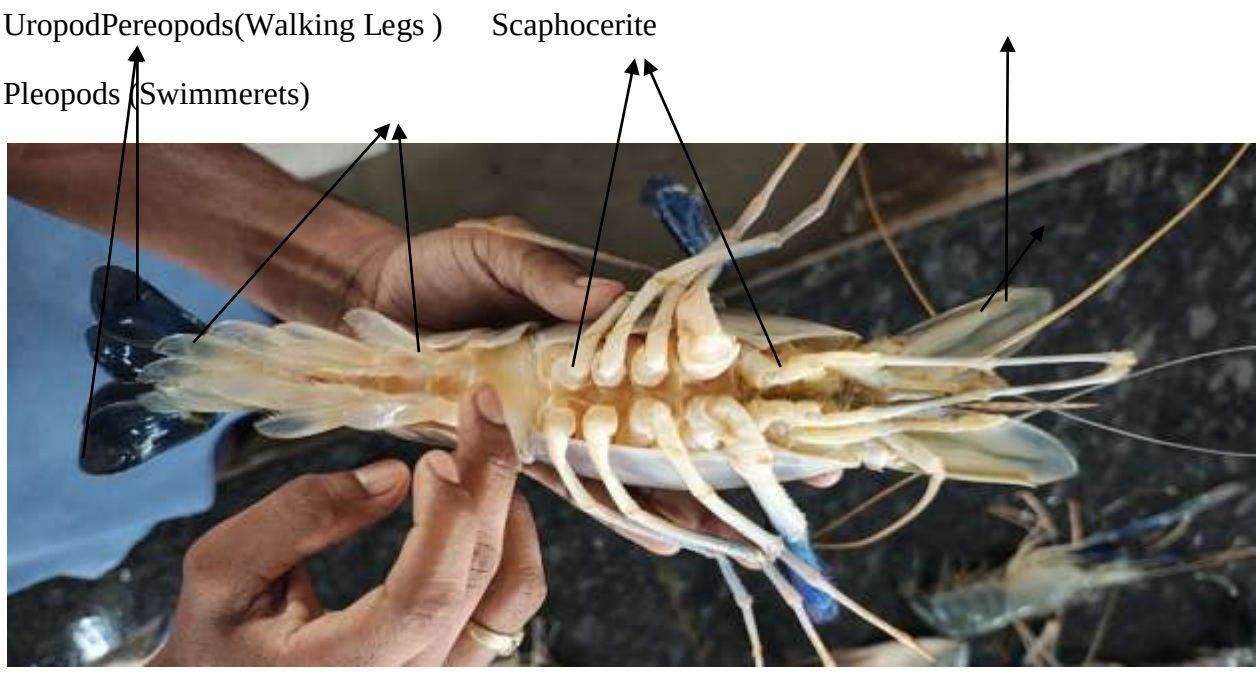

Fig : - 3 *M.rosenbergii*

Morphological Identification Of selective specie *M.rosenbergii*

- Cephalothoraxand abdomen (25) cm body divided into 20 segments 14 segments in head and 8 segments in abdomen

- Cephalothoraxes (head - 10) cm front part of cephalon 6 segments (Rostrum-10 cm long slightly curved upward wit 14 dorsal teeth and 12 ventral teeth ; eye-1 cm maxillae 5 to 8 cm) and (5 pare of appendages)
- Rear part thorax 8 fused segments 3 set of maxillipeds – 10 to 45 cmand 5 pare of pereopods-15 cm (walking legs)
- Abdomen (tail - 15) cm 6 segments each bearing 5 pair of appendages known as pleopods-1.5 cm (swimming legs)
- Uropod- 4 cm
- Telson - 3 cm

Identification of male and female prawn

Future	**Male prawn**	**Female prawn**
Figure		
Body size	Large and robust	Small and slender
Color	Greenish to brownish -gray	Light greenish to brown -gray
Second chelipeds (claw)	Very large prominent ; long and strong color variations	Short and slender
Rostrum	Longer ; slightly curved	Slightly shorter than males
Abdomen segments	Straight	Border abdomen for egg carrying
Genital pore (located)	At the base of 5th walking leg (pereiopod)	At the base of 3rd walking leg (pereiopod)
Pleopods (walking legs)	Smaller , mainly used for swimming	Large and feathery to carry eggs
Reproductive organs	Presence of appendix masculine on the second pleopod	Absence of appendix masculine
Egg presence	No eggs	May carry eggs under the abdomen (berried female)

Fig :- 4,Giantfreshwaterprawn *Macrobrachium rosenbergii* (Male & Female)

Classification of External Morphology ofGiantfreshwater Prawn *(Macrobrachiumrosenbergii)*

BODY SECTION	SEGMENTS	APPENDAGES NAMES (PAIRS)	FUNCTIONS OF APPENDAGES AND RELATED STRUCTURES
Cephalon front part of the cephalothoraxes	1	Embryonic segment (not visible in adults)	
	2	1st Antennae	Tactile for sensory perception
	3	2nd Antennae	Tactile
	4	Mandibles	Cutting and grinding food
	5	1st Maxillae	Food handling
	6	2nd Maxillae	Food handling ; water circulation through the gill chamber
Thorax rear part of the cephalothorax	7	1st Maxillipeds	Feeding /food handling
	8	2nd Maxillipeds	Feeding /food handling
	9	3rd Maxillipeds	Feeding /food handling
	10	1st Pereopods	(Chelipeds) food capture
	11	2nd Pereopods	Food capture ;fighting behavior during mating
	12	3rd Pereopods	Walking and female gonophores between legs
	13	4th Pereopods	Walking
	14	5th Pereopods	Walking male gonophores between base of legs
Abdomen	15	1st Pleopods	Swimming legs
	16	2nd Pleopods	Swimming legs
	17	3rd Pleopods	Swimming legs
	18	4th Pleopods	Swimming legs
	19	5th Pleopods	Swimming legs
	20	Uropod	Locomotion and stability

Dissection Of Selective SpeciesGiant Freshwater Prawn *M.rosenbergii*

Fig :-5 Dissection of prawn *M.rosenbergii*

The above figure indicating dissecting the prawn on the day of experiment in CRL (central research laboratory)and extracted the Gut from selective species Giant freshwater prawn *(Macrobrachium rosenbergii)*

Internal Anatomy of Giant Freshwater Prawn *M. rosenbergii*

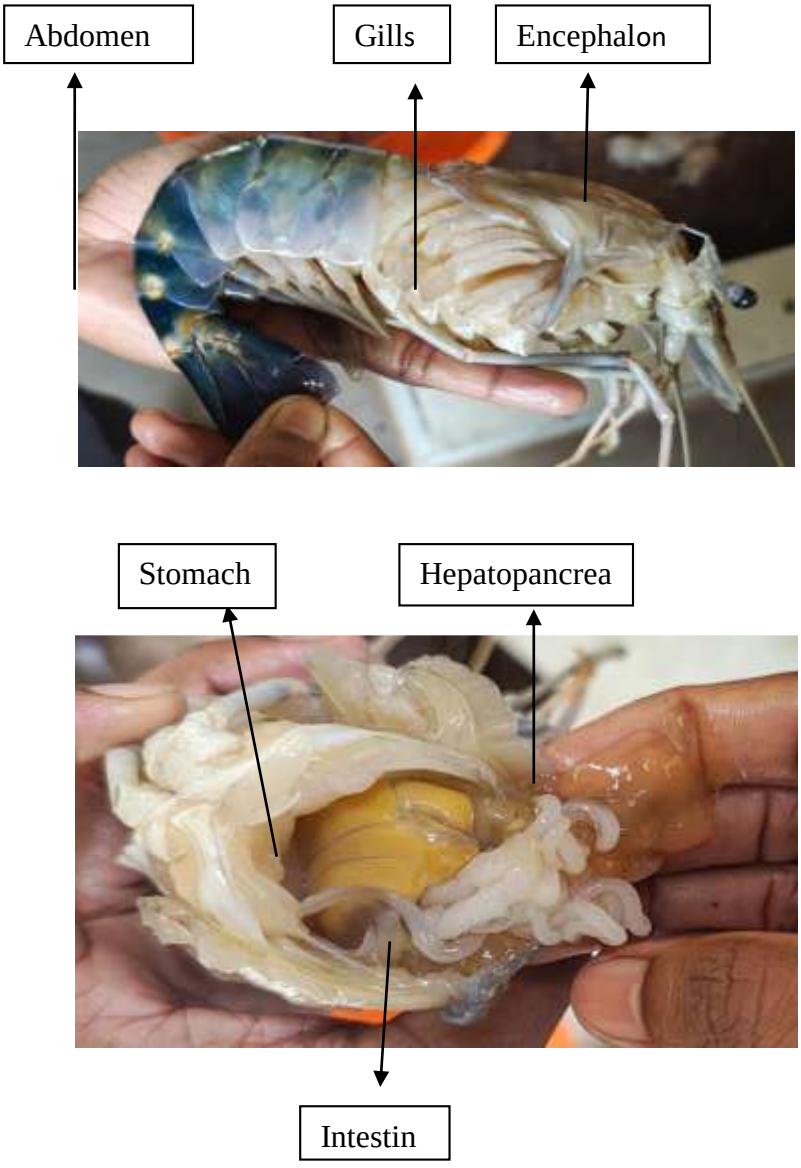

Fig :- 6,(Digestive Track) Gut of*M.rosenbergii*

2. TO ISOLATE THE BACTERIA FROM GUT OF GIANT FRESHWATER PRAWN

(*Macrobrachium rosenbergii*)

1.1 SPREAD METHOD FOR PURE CULTURE

Culture media preparation :-

- Primarily we have to weigh the sufficient amount of Nutrient Agar and Agar -Agar powder
- Then calculate the amount of NutrientAgar media necessary for the 125 ml distilled water
- For 1000ml water needs to dissolve 28 gramsNutrient Agar powder
- 28 grams /1000 ml *125ml = 3.5 grams Nutrient Agar powder we need
- Now we should weight balance as 3.5 grams ofNutrient Agar and 1 gram of Agar – Agar powder
- Then add the power into conical flask capacity 500ml then add 125 ml of distilled water into the flask after addition of water mix properly so all the clumps of the powder will get properly dissolved in water
- In the next step we are going to make cotton plug for non-absorptionCeliac so cotton plug the conical flask properly.

Fig :- 7, Weighting media

Fig:-8,Autoclave setting

Fig :-9 Autoclaving

Autoclaving keeps the flask in the autoclave for 15 minutes 121℃ at 15 Lbs. pressure up to autoclave

When the pressure comes “0” then open the lid slowly and allow it to cool down

Serial dilution :-

Serial dilution method is one of the most common techniques for the isolation of pure culture

It is a step wise dilution of sample in solution to reduce high concentration solution in dilution solution

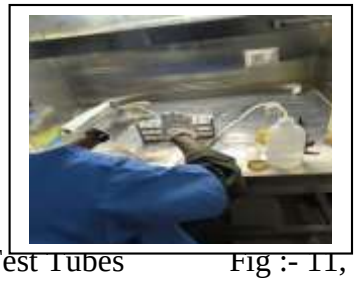

Fig ;- 10,Label Test Tubes

Fig :- 11, Serial Dilution

- Then Label test tubes (10^{-1} to 10^{-10}) and fill them as 9ml of distilled water in each test tubes indicating factor aseptically add 1ml of enrichment sample to the first test tube ($10^{-1)}$ and mix gently
- Take 1ml of this dilution and add to next test tube (10^{-2}) mix gently
- Repeat this procedure for remaining test tubes (10^{-3} to 10^{-10})
- In this serial dilution we have to take o.1 ml of sample from last (10^{-6} to 10^{-10}) diluted test tubes add this 0.1 ml of sample on petri plates

Spread plate method :-

Before going to labeling clean all the equipment's with 99% ethanol , because ethanol ,up to 99% concentration ,prevents microorganism it can be used to disinfectant lab surface and equipment's put them in UV chamber under UV light leave it for 15 minutes

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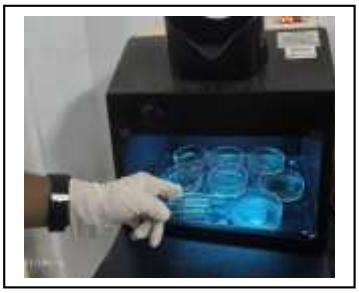


Fig :-12, UV chamber

Fig :-13, label all equipment's

Prepare a suitable area as laminar air flow for the pouring and label media

Then label all the petri plates as serial number (1 to 5) name the plate as (Eg :- sample -1) pour approximately 20 to 25 ml of media into eachNutrient Agar petri plates slightly lifting the petri plate from left one side pouring should cover the base of the plate immediately cover the petri plate keep a side repeat the procedure and pour into the remaining petri plates in the similar way

Fig :- 14, Pouring media Fig :-15,Labeled Nutrient Agar Plates

After then take 0.1 m of sample from each (10^{-6} to 10^{-10}) diluted mixture with micropipette then placed on the surface of each Nutrient Agar petri plate

Fig :- 16, Inserting diluted mixture Fig :-17, Stirring

Now the next step spread evenly over the surface by using (L shape glass rod) before using the glass rod heat it over the flame 5 seconds heating and cool down it for 5 seconds then use it for stirring the solution surface of Nutrient Agar and immediately cover the Petri plate and leave it for 30 to 40 minutes

In the next step , when the Nutrient Agar media were in the Petri plates were dry are solidified

Incubate media petri plates should put them in invert position at 37℃ for 24 hours .

Fig :-18, Incubating in Invert position

Then next day after 24 - 48 hours then observe the isolated culture media petri plates .

On that petri plates found cream color likebacteria colonies were formed on that Nutrient Agar media

Fig :- 19, Pure cultured Nutrient Agar petri plates

On the above culturedplates, I have selected low colonies Petri platefrom of those 5 cultured plates as (sample named 10^{th}) for further culture as sub culture

Fig :- 20,Bacterial cultured plate with (~ 30 colonies)

1.2 SUB-CULTURE FOR STREAK METHOD

Sub-culture used for transferring bacteria colonies from one existing culture to a fresh growth media

This process is essential for maintaining bacteria culture and it involves transferring a small sample of the original culture

CFU (colony forming unit) is to count and estimate the number of living and able to grow microorganisms in a sample .each colony forming unit represent one cell or group of cells that can grow into a single colony on aNutrient Agar plate

In This colony forming unit/ per ml we can accurate how many living bacteria were present per 0.1 ml in our original sample (undiluted sample)

per ml of originaldiluted sample and number of colonies presented for 0.1ml sample

colony count range :-

We observed about ~ 30 Colonies in a sample 10^{th}

Dilution taken from test tube 10^{-10}

Volume of plated = 0.1 ml

Colony count technique :-

Valued plate count is between ~ 30 to 300 colonies

More than 300 colonies – TNTC (Too numerous to count)

Fewer than 30 colonies – TFTC (Too few to count)

For accuracy choose a plate with 30 – 300 colonies

Colony count example :-

Calculation of CUF/ML using colony count

(Cfu /mL = Number of Colonies X Dilution Factor / volume plated in ml

0.1 ml from tes tube (10^{-10}) dilution ~ 30 colonies

CFU/mL =30 x 10^{-10} /0.1 =~3000

Cfu/mL (colony-forming unit per mL= ~3000)

Streak Plate Method of Isolation :-

Again, freshly prepare Nutrient Agar media for sub culture

It is an isolation procedure used for obtaining a streak plate method of sub culture

Prepare Nutrient Agar media and then choose a suitable Ares as laminar air flow for the pouring and labeling Nutrient agar petri plates and leave it for 30 to 40 minutes

- In the next step take a dry Nutrient Agar plate for streak plate method
- Then flame inoculation wire loop hold the wire loop in the flame for a few secondsuntil it appears reddish color
- After flaming allow the wire loop to cool down completely
- Then the loop is sterile and you can take out culture sample using this inoculation wire loop
- Now dip the wire loop into the culture sample and take out a loop full of the bacteria sample
- Place the wire loop containing sample near the edge of the sample petri plate in area and streak back forth in a zig zag motion to spread culture uniformly

Then after now flame the wire loop again and allow it to cool and rotate the Petri plate in 90°

Degrees anti clock wise

Now touch the few lines in the corner of area 1 and drag the wire loop into area 2 and streak back and forth in zig zag motion to spread culture homogeneously

Fig :- 21, Streak plate method

Again, rotate the petri plate at 90°degrees anti clock wise

Now drag the loop from corner of area into area 3 and streak back and forth in area 3

Finally heat wire loop and keep a side

Now close the lid of petri plate and keep the petri plates in incubator at 37℃ degree Celsius for 28 to 48 hours for incubation

In the next step after 48 hours incubation remove the petri plates from the incubator and check the growth of bacteria on the Nutrient Agar media

Fig :-22, Sub cultured plates

3. TO IDENTIFY THE TYPE OF BACTERIA STAIN PRESENT IN GIANT FRESHWATER PRAWN *(Macrobrachium rosenbergii)*

Gram staining procedure:- This culture is spread with an inoculationloop to an even thin film over a circle of 15mm in diameter a typically slide can accommodates up to 4 small smears

Select Colony from The Culture of Nutrient Agar Plate

Prepare Bacteria Smear on Slide

⬇

Then heat the Slide (Gently Pass Over the Flame)

⬇

Now Add Crystal Violet Stain (Primary Stain)Wait For 1 Minute

⬇

Rinse It with Distilled Water

⬇

Then Next Add Grams's Iodine Solution (Mordant)Wait For 1 Minute

⬇

Again, Rinse It with Distilled Water

⬇

Decolorize With 99% Alcohol For 10 To 20 Seconds

⬇

After That Immediately

⬇

Then Last Add Safranine For (Counterstain)Wait For 1 Minute

↓

Then Observe Slide Under Microscope

Fig :- 23,Picking colony from sub-culture plate

Fig :- 24, Prepare Bacteria Smear on Slide

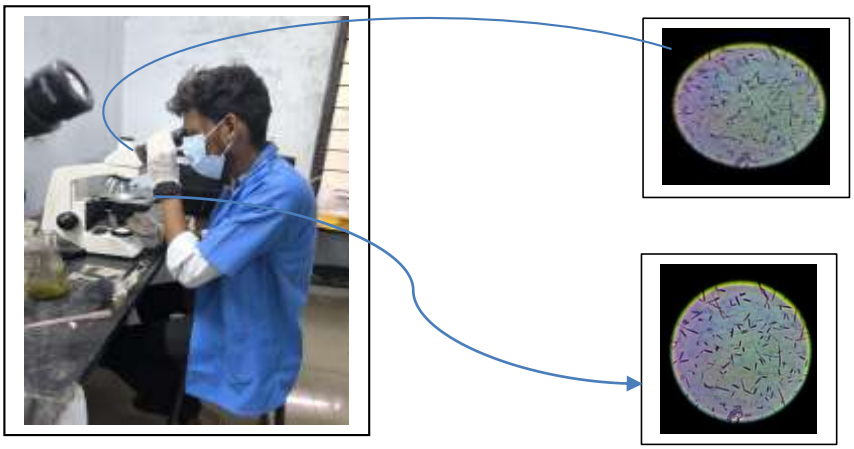


Fig :-25, Microscopic examination

Microscopic Examination and Observation ofBacteria Stain :-

- **GRAM POSITIVE BACTERIA :-**

They were Retain the crystal violet and appeared Purple and Blue color

- **GRAM NEGATIVE BACTERIA :-**

They were not retaining crystal violet , but are count stained with safranin appeared Pink and Red color

Gram – positive and gram-negative bacteria both can exhibits various shapes

IDENTIFIED SHAPES :-

Cocci –round or spherical

Bacilli –rod –shaped

Vibrio – comma shaped

Coccobacilli – short oval rods

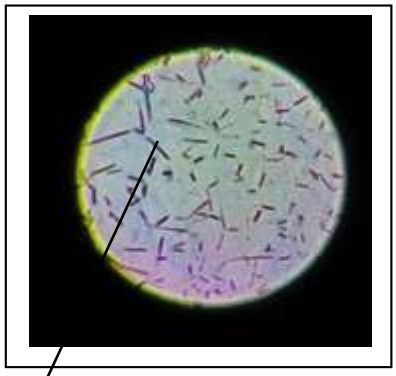


Fig :-26,Shape of bacteria colony

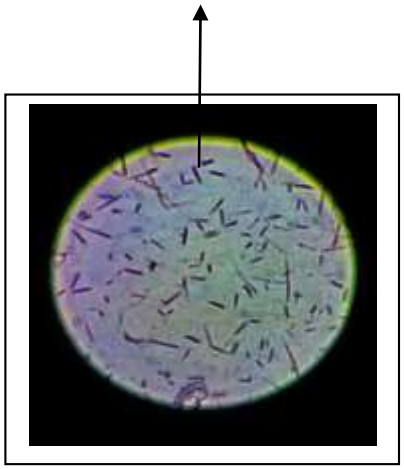


Fig :- 27,Shape of bacteria colony

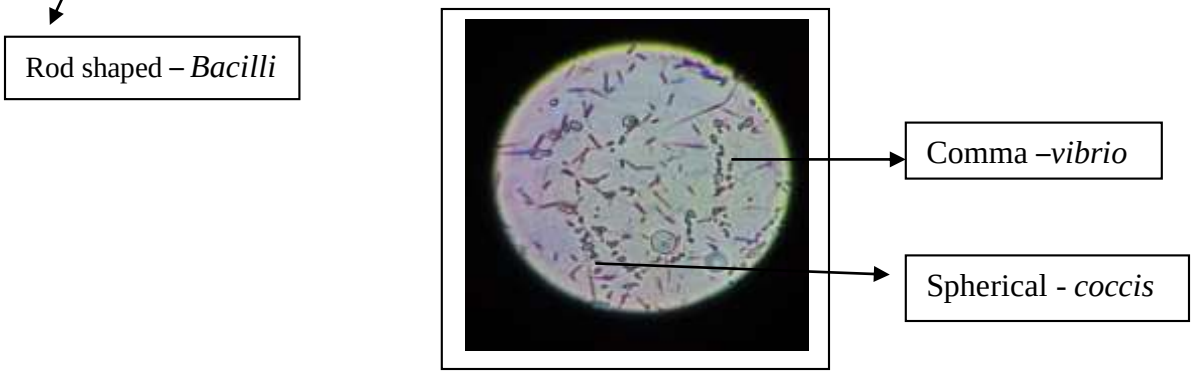


Fig :-28,Shape of bacteria colony

RESULT :-

Gram staining and microscopic examination :-

Gram – Positive and gram-negative bacteria observed and identified among the isolates 60% were Gram – Positive bacteria rod ,short oval rods and 40% were Gram Negative Bacteria *cocci* and rod comma etc. and these shaped bacteria were predominant in gut sample of Giant freshwater prawn *(Macrobrachium rosenbergii)* .

Morphological characterization of bacteria isolates

Rod-Shaped Bacteria (*Bacilli*)

These bacteria have a cylindrical or elongated shape, resembling rods. They are commonly referred to as bacilli (singular: bacillus).

Rod-shaped bacteria:

- *Escherichia coli*
- *Bacillus subtilis*
- *Lactobacillus spp.*
- *Pseudomonas aeruginosa*
- *Salmonella spp.*

Key features:

Shape: Cylindrical or elongated

May occur singly or in chains

Cocci or Coccoid Bacteria

These bacteria are spherical or oval-shaped, commonly referred to as cocci (singular: coccus).

cocci bacteria:

- *Staphylococcus aureus* (grape-like clusters)
- *Streptococcus pyogenes* (chains)
- *Enterococcus faecalis* (pairs or short chains)
- *Micrococcus luteus* (tetrads or clusters)

Key features:

Shape: Spherical or nearly spherical

Can be arranged as:

- Diplococci (pairs)
- Streptococci (chains)
- Staphylococci (clusters)
- Tetrads (groups of four)

Fig :- 1 Gut Sample

Fig :- 2 Autoclave

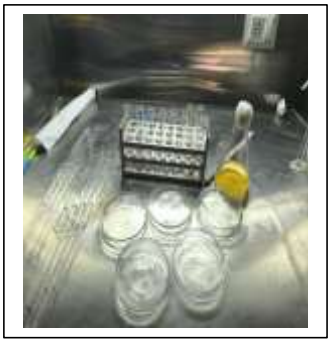


Fig :- 3 Labeling Plates

Fig :-4 Serial Dilution

Fig :- 5 Inserting Sample

Fig:- 6 Spread Method

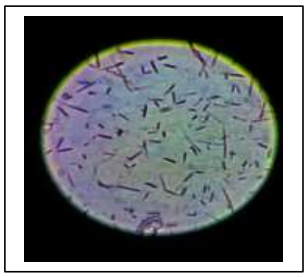
Fig :- 7 Incubating

Fig :- 8 Pure Culture

Fig :- 9 Steaking Method

Fig :-10 Sub Culture

Fig :- 11 Bacteria Smear Slide Fig :- 12 Microscopic examine



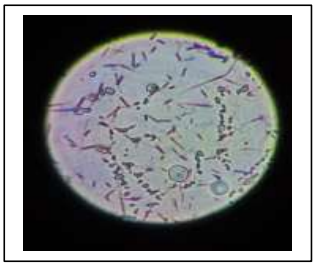


Fig :- 13 Shape of Bacterial colony Fig :- 14 Shape of Bacterial colony , Fig :- 15 Shape of bacterial colony

DISCUSSION

The result of this study presents precedent information on the typical gram bacteria associated with Gut of *Macrobrachium rosenbergii*. the majority of the types of bacteria stained were Gram-negative and included *Aeromonas, Pseudomonas, Vibrio,* and *Bacillus* species. *Staphylococcus aureus* and *Escherichia coli* were the two most prevalent Gram-positive bacteria. The research presented here details the bacterial stain found in Gut of Giant fresh water prawn *Macrobrachiumrosenbergii*

Gram staining identified both Gram-positive *cocci* and Gram-negative rods. Gram-negative bacteria with rod-like shapes were predominant particularly *Vibrio* and *Aeromonas* species, however *Micrococcus* and *Enterococcus* species were Gram-positive *cocci*. According to (Lavilla-Pitogo et al. 1990), Gram-negative organisms were similarly prevalent among *crustaceans,* indicating that they were able to live in watery habitats.

The bacterial flora found in the digestive system of freshwater prawns raised and sold in the current study in(1970, Vanderzant et al). reported that *Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter* and *Aeromonas,* and *Vibrio ssp* were the dominant flora in a pond-reared system, while *Macrobrachium rosenbergii* demonstrated *Escherichia coli* as the predominant bacteria, a reflection of the bacterial flora of the water as proposed by Roberts in 1978. This variance may be due to sampling methods and geographic variations where the entire prawn or different sections had been used as the sample.

In prawns that were consuming the naturally occurring substances rich in bacteria from mud substrates as well as the *epifloral* and *epifauna*, Doriarty (1976) and Dale (1968) noted a larger bacterial population and the bacterial genus in the stomach contents. When pond-reared prawns were fed pelleted feed as an additional diet, Moriarty (1976) observed that the majority of the feed was promoting healthy bacterial development. As a result, Vibrio may act as opportunistic infections that invade tissues and hemolyze through the intestinal wall when the prawns are exposed to them, as proposed by Davis and Sizemore (1982).

CONCLUSION :-

The study successfully isolated and identified a range of gram-positive bacteria and gram – negative bacteria associated in gut of Giant freshwater prawn *Macrobrachium rosenbergii*

Gram – positive and gram -negative bacteria both can exhibit various shapes

Identified shapes :-

- *Cocci* – round or spherical
- *Bacilli* – rod shaped
- *Vibrio* – comma shaped
- *Coccobacilli* –short plum rods

Rod-shaped bacteria:

- *Escherichia coli*
- *Bacillus subtilis*
- *Lactobacillus spp.*
- *Pseudomonas aeruginosa*
- *Salmonella spp.*

Cocci Bacteria:

- *Staphylococcus aureus*
- *Streptococcus pyogenes*
- *Enterococcus faecalis*

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