"Isolation, Identificationand Assessmentof Organic Carbon Degradation Potentialof Zooplankton Associated Bacteria"

A Dissertation for

MIC- 651 DISCIPLINE SPECIFIC DISSERTATION

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Submitted in partial fulfilment of Master of Science in Microbiology

By

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DECLARATION BY STUDENT

I hereby declare that the data presented in the dissertation report entitled, "**Isolation, Identification, and Assessment of Organic-Carbon Degradation Potential of Zooplankton associated Bacteria**" is based on the results carried out by me, in the microbiology department at the School of Biological Sciences and Biotechnology, Goa University under the supervision of Dr. Lata Gawade. I further declare that the work reported in this project has not been submitted and will not be submitted elsewhere for the award of any other degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation "**Isolation, Identification and Assessment of Organic-Carbon Degradation Potential of Zooplankton associated Bacteria**" is a bonified work carried out by **Ms. Kareena L. Velip** under my supervision/mentorship in fulfillment of the requirements for the award of the degree of Master of Science in the Discipline Microbiology at the School of Biological Sciences and Biotechnology, Goa University.

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PREFACE

Estuaries receive tons of organic matter throughout the year. This in the long run contributes to carbon cycling. Recently carbon cycle has received attention as carbon in various forms affects life on Earth. Carbon in the form of CO_2 gas acts as a heat trapper that heats the Earth's atmosphere contributing to global warming.

In view of the requirements to understand the role of zooplankton-associated bacteria in organic carbon degradation and nutrient cycling, the current study focuses on the need to solve the problem of organic carbon that is released in the environment through various human and natural activities by exploring the potential of zooplankton associated bacteria in doing so.

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ABSTRACT

Release of carbon is a major issue nowadays. Sources that contribute are of mainly man-made origin. Estuaries are a storehouse of organic matter that it receives from yearly river runoffs. This release affects the carbon cycle in the aquatic environment. However, the utilization of this organic matter by various means is crucial and important.

Zooplankton and zooplankton-associated bacteria play a major role in the cycling of this matter. Zooplankton provide a microhabitat for these microbes to grow and they can utilize the form of organic carbon that is released into the water bodies.

The current study focusses on isolating and finding zooplankton associated bacteria with a potential to degrade organic carbon. As utilizing the natural ability of zooplankton associated bacteria is a environment friendly way of to help us deal with massive amounts of organic carbon that is out in the environment.

A considerable number of zooplankton associated bacterial cultures were isolated from zooplanktons and their potential in carbon degradation was checked using the respiration rate of the isolates in natural and artificial carbon substrate. The isolated cultures were also screened for their amylase activity.

1. INTRODUCTION

The bacterial community plays a very crucial role in all the aspects of life which is connected to the flora and fauna living on the planet Earth. Giving their contribution in various biological processes such as the carbon cycle, nitrogen cycle, phosphorous cycle, and so on. Bacteria play a central role in the oxidation of organic matter. Bacterial metabolic rates and carbon modification are regulated by physicochemical changes mainly influenced by the annual monsoon river run-off.

Estuaries receive organic matter (OM) from multiple sources, including terrestrial C3 and C4 plants, soil, freshwater, estuarine and marine phytoplankton, and domestic industrial sewage. The contribution of OM from diverse sources makes the OM cycling in estuaries more complex. Biogeochemical cycling of OM in aquatic systems is of global concern as it strongly influences the global carbon cycle by acting as a sink or source for atmospheric carbon dioxide (CO2). Being highly productive zones estuaries can act as a sink, or due to extensive processing of OM, they can be a source of atmospheric carbon dioxide. For example, the Indian estuaries, Godavari (pCO2: 221-34016uatm; Sarma et al. 2011), Hooghly (220-1200uatm; Mukhopadhyay et al. 2003), Mandovi (110-2300uatm; Sarma et al. 2001), were reported as a seasonal source of CO2 to the atmosphere. (Krishna et al. 2015).

In many ways carbon is life. A chemical element, like nitrogen or hydrogen, carbon is a basic building block of biomolecules. It exists on Earth as solid, dissolved and gaseous forms. Carbon-dioxide traps heat produced both in nature and by human activities. Human contribution to Carbon dioxide is mainly through burning of fossil fuels such as coal, natural gas and the exploitation of oil used in the power generation and transportation. Also, released through land use and forest fires. This buildup of Carbon dioxide can lead to heat trapping and contribute to climate change. Due to changing climatic conditions and increase in atmospheric

CO_2 concentrations, the carbon cycle has received much attention. Attempts have been made to understand the underlying mechanisms involved in carbon modification and transformation. Heterotrophic bacteria play crucial role in modifying and mineralizing the organic carbon, thus introducing it to the aquatic food web (Pomeroy 1974; Azam et al. 1983). During the bacterial mineralization, part of organic carbon gets converted to biomass (production), and rest gets respired as inorganic carbon i.e. CO_2 (Hopkin and Smith 2005). Respiration of organic carbon in the estuarine system represents a return of CO_2 to the atmosphere previously fixed by the terrestrial systems (Richey et al. 2002; Cole et al. 2007). In aquatic systems, these modification of organic matter by bacteria are been affected by temperature (Lopez and Moran 2007; Rivkin and Ledgender,2001), salinity pattern (David et al. 2000; Crump et al. 2004), light availability, pH changes, turbidity (Roland and Cole,1999), quality of the organic matter available (Reinthaler et al.,2005; Apple et al.,2006; Ylla et al.,2012) and bacterial community composition (Logue et al. 2016). These factors have a significant impact on bacterial mineralization of organic carbon, particularly in estuaries. Despite of bacterial contribution in carbon cycling and Biogeochemical processes, studies on measurement of their metabolic rates on changing nature and source of organic matter and relation to surface water pCO_2 levels are scarce.

Polysaccharides are abundant macromolecules that are found in all ecological habitats. Polysaccharides are found in varying forms and complexities along with their role as store house of photosynthetically fixed carbon source of energy utilized by the various organisms for their metabolic machinery. In bacteria, the genes encoding these specific proteins are grouped together in the genome in coregulated regions known as polysaccharide utilization loci (PULs). These PULs define their metabolic capacity to utilize polysaccharides and contribute in determining its environmental niche.

The current study is carried out with an aim to isolate, enumerate and assess the organic carbon degradation potential of zooplankton associated bacteria, by analysis of the metabolic rates of selected isolated culture and also screening the cultures for their enzymatic activity.

Zooplankton offer a microenvironment to a large number of bacterial communities. Usually, highest percentage is seen of bacteria belonging to the *Vibrio spp*. As per the reports, 0.01% to 40% of the water column bacteria is known to be associated with zooplankton. The zooplankton associated bacterial composition vary according to different species. A growing admiration in marine microbial ecology is been seen, especially in the case of particle associated bacteria because it provides them microhabitat for colonization. Bacterial abundance is less in open oceans than in coastal, and estuarine systems, where there is high number of particles and likely higher percentage of particle associated bacteria. These might be metabolically very active and can play a significant role in organic carbon degradation and sequestration. (Heidelberg et al.,2002).

1.2. HYPOTHESIS
1.3.AIM AND OBJECTIVES

1.2. HYPOTHESIS:

Zooplankton associated bacteria will be highly efficient in carbon degradation.

1.3.AIM: To isolate, enumerate and assess the organic-carbon degradation potential of the zooplankton associated bacteria.

OBJECTIVES:

- Isolation and enumeration of zooplankton-associated bacteria
- Identification of the bacterial isolates
- Screening the isolates for enzyme activity i.e. amylase, cellulase
- Assess the potential for degradation of natural and artificial carbon substrates of selected microorganisms.

2.LITERATURE REVIEW

2.1. THE CARBON CYCLE

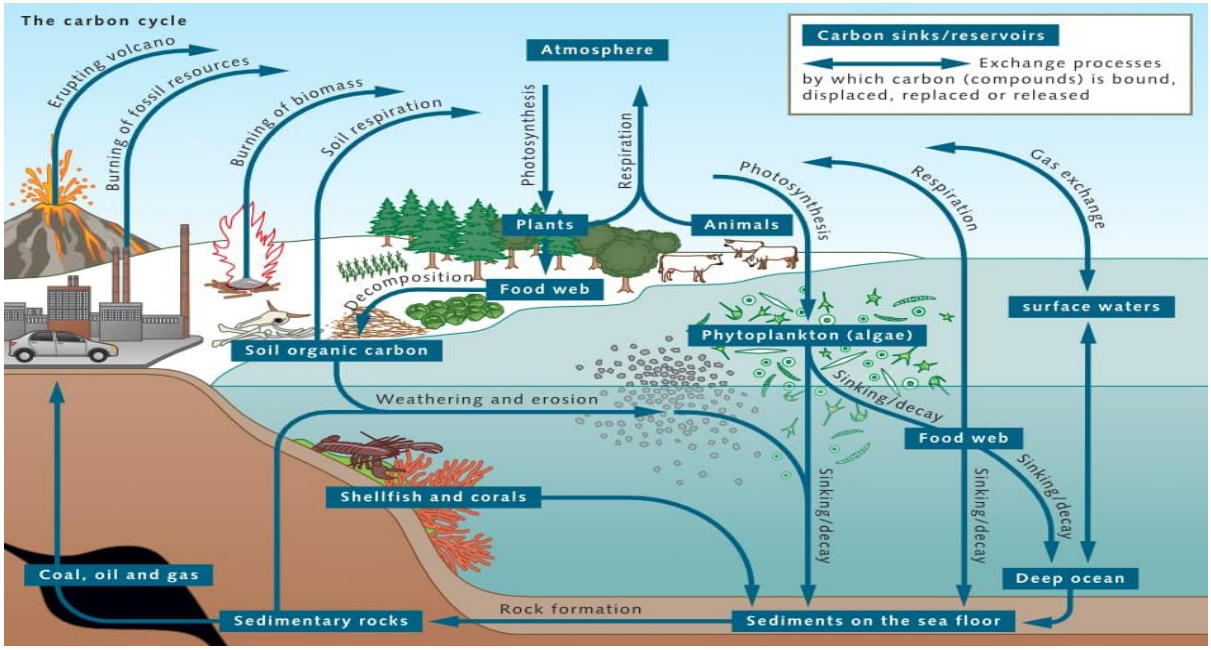


Fig no. 2.1: THE CARBON CYCLE (Erven, 2024 - https://images.app.goo.gl/a4FxEFV6pPLPPPEi8)

Carbon is the backbone of all living things on the planet Earth. It is present in rocks, sediment, cells and even our genetic makeup has carbon. Carbon does not remain in one it moves from one reservoir to another in instants, this is caused by the natural and human processes that occur in the entire world. This can be collectively termed as the Carbon cycle. The largest water body i.e. Ocean plays a very crucial role in the entire Carbon cycle and nutrient cycling.

Changes in the food supply to deep oceans are usually related to benthic microbial community structure along with the functioning of the ecosystems (Danovaro and Loreau,2008). Because these communities seem to respond to food supply with changes in abundance, community structure, remineralization of carbon, and ecosystem function. The pervasive climate changes foreseen by the Intergovernmental Panel on Climate Change (IPCC) may have lasting impacts on deep sea also (Solomon et al.,2007).

Organic matter utilization is been measured in situ using the autonomous respiration checker instrument in the sea bed. The term usually used for this is sediment community oxygen consumption. Long time-series records have shown a strong seasonal signal in SCOC, which is highest in summer and fall and lowest in winter at the Northeast Pacific site and believed to be driven primarily by microbes and smaller fauna (Drazen et al.,1998; Ruhl,2008). Larger fauna have been observed to mix fresh Phyto detritus centimeters into abyssal sediments, thus increasing the likelihood of carbon burial and facilitating aerobic life deeper into the sediment. Such facilitation is thought to explain the recently observed positive relationships between deep-sea biodiversity and ecosystem functioning (Loreau,2008).

Over an extended time- series in the Northeast Pacific, seasonal fluctuation in SCOC was in relative synchrony with the food supply but displayed less interannual variability. However, there are records of discrepancy between food supply and utilization, with decreasing supply of POC to meet needs of the benthic community. The ratio of POC flux to SCOC (POCF/SCOC) showed declined by one unity in 1989 to 0.2 in 1996. The ratio rebounded to 0.4 in 1998 (Smith et al.,2001) but remained below unity from 2005 to 2007 after a 5-year hiatus in measurements. The deficiencies in food chain supply in the ocean can be combated to some extent by utilizing the standard stock of organic carbon in the benthos (Smith et al.,2001). It is possible that a portion of this deficit in food supply results from the under sampling by sedimentation traps of sinking POCF in the form of discrete detrital aggregates (Lampitt et al., 2001; Baldwin et al.,1998; Robison et al., 2005). However, when POCF is directly measured after supplementing carbon fluxes based on visible sea-floor aggregates, there is still a deficit in the available food supply (Smith et al., 2008). The quality of the available food with long-term deficits is thought to change, with less fresh POC. Fluctuations in food supply driven by climate variation ultimately are linked to changes in benthic community structure and processes: higher POCF is significantly correlated with increased

SCOC on both seasonal and interannual time scales (Ruhl et al., 2008). In particular, the deficiency in the particulate flux can impact biogeochemical and ecological relationship, that can affect the ocean functioning in future. It is possible that the noticeable difference between sinking food supply and benthic community utilization can be reconciled by episodic inputs of organic matter in the oceans/ estuaries for that matter.

2.2. ZOOPLANKTON AND ASSOCIATED BACTERIA

Carbon usually flows from the pelagic ecosystems and is very complicated and functionally extremely diverse. At all the extremes the, zooplankton-mediated food web linkages help in the nutrient cycling that sustain high level of productivity in open ocean. Their contribution is through different mechanisms of mucous feeding webs, fecal pellets, molts and vertical migration (Steinberg and Landry, 2017)

Crustacean zooplanktons release a copious amount of particulate organic matter in the oceans (Heinle et al.,1997). Microbes associated with the zooplankton use them as carbon and nutrient enriched habitat. The gut of zooplankton provides them with hypoxic environment that facilitates the marginal but a very important anaerobic processes such as denitrification, dissimilatory nitrate and nitrite reduction and methanogenesis in the ocean (De Angelis and LEE,1994; Stief et al.,2017).

In the zooplankton microhabitat, genes indicative for surface attachment and encoding for pili, fimbriae and chitin-recognition proteins are used to colonize the gut and external surface (Bodelon *et al*.,2013). The high number of glycosyl hydrolase encoding genes (mainly associated with the *Flavobacteria clade*, suggests the capability of the zooplankton□associated bacterial community to metabolize polysaccharides and amino□sugars, such as cellulose or chitin respectively (Beier and Bertilsson, 2013). *Flavobacteria* have been shown ability to

utilize chitin and N□acetyl glucosamine (Cottrell and Kirchman, 2000). Hence, through this study it confirms that crustacean zooplankton and *Flavobacteria* are in association. They help the zooplankton to metabolize organic matter from its exoskeleton. *Vibrio spp.,* are other important player in chitin mineralization often found in association with crustacean zooplankton (Erken et al., 2015) as shown in previous studies conducted in coastal systems (Montanari et al., 1999; Turner et al., 2009). This discrepancy could be explained by a lower abundance of *Vibrio ssp*. in cold ocean waters as compared to warm coastal area (Vezzulli et al., 2012). Additionally, they also show amylase, and pectinase activity that confirms they are utilizer of starch, pectin derived from the zooplanktons (Moal et al.,1987; Alderkamp et al., 2007).

Metagenomic and proteomic studies revealed that taurine might be a substrate for heterotrophic marine bacteria (Poretsky et al., 2010; Sowell et al., 2011; Williams et al., 2012). The importance of it been demonstrated using SAR11 cultures (Carini et al.,2013). The concentration of dissolved taurine in the ocean is been increasing (Clifford et al., 2017). Taurine is organo-sulphonate found in tissues of marine zooplanktons and are potential carbon, nitrogen and Sulphur for bacterial community (Williams et al., 2012; Carini et al., 2013). Thus, the zooplankton□associated bacterial community is found in close proximity to its body which is a source of Taurine. The presence of taurine catabolic genes in the metagenomes such as the taurine□pyruvate aminotransferase and Sulfur□acetaldehyde acetyltransferase indicates the potential importance of taurine as a substrate for zooplankton□associated bacterial communities. Surprisingly, even though most of the taurine catabolic genes were associated with Alpha-*proteobacteria*, none were affiliated to SAR11, likely due to the low contribution of this clade to the zooplankton□associated bacterial community.

The copepods hindgut provides a habitat with low oxygen concentration, and low pH; Which suggests that their gut is suitable for the growth of anaerobic microbes that can tolerate acidic

conditions (Tang et al., 2011). There are metagenomic insights that show zooplankton-associated bacteria harbor's genes that can regulate cytosolic acidity by removing protons or using ammonia as scavengers (Booth, 1985; Slonczewski et al., 2009).

Thus, the zooplankton□associated bacterial metabolic pathways could play a crucial role in the recycling of iron through grazing of diatoms in iron limited regions (Hutchins and Bruland, 1994; Hutchins et al., 1995).

2.3. ROLE OF AQUATIC BACTERIAL COMMUNITY IN DEGRADATION OF DISSOLVED ORGANIC MATTER

Oceans dissolved organic carbon content is as much as present in the atmosphere. Marine DOC is a large reservoir of 660 Pg C, that plays a role in the ocean food web and similarly interacts with the climate in long run. Due to this reason Carbon cycle is receiving attention now a days. Many attempts are been made to study the entire process and how to combat with the release of carbon, the main reason being the human exploitation of fossil resources, crude oils, and industrial dumping in the oceans.

Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter, showcase that going beyond the bacterial community composition and Dissolved organic matter degradation, their result shows that the 4 experimental communities degraded different substrates from the DOM pool (Logue et.al, 2016).

As per recent studies, Organic carbon in rivers is estimated to be 10.4mg C/L. and hence annual input in the ocean is reported to be $3.0*10^{14}$g C. The global input varies between 0.8-159.0mg/L (Liu and Wang,2022).

3.MATERIAL

AND

METHODOLOGY

MATERIALS AND REQUIREMENTS

GLASSWARES: Petri plates, test tubes, conical flasks, beakers, glass rods, slides, L-shape glass spreaders, quartz cuvettes, burette, DO bottles, glass pipette, etc.

CHEMICALS: $MnSO_4$ solution 1M, Alkali iodide 1M, $Na_2S_2O_3$ stock0.75 N, Conc.H_2SO_4, ethanol, Gram Crystall violet, Gram's iodine, safranin, methyl red indicator, 1% starch solution, 1% tetra-methyl-p-phenylenediamine dihydrochloride, Bromothymol blue, Kovac reagent, Phenol red, H_2O_2 solution, starch soluble, Carboxymethyl cellulose, etc.

MEDIA: Zobell marine agar, tryptone broth, Glucose peptone broth, Simmons Citrate agar, Christensen's urea agar, Triple sugar iron agar, Nutrient agar

1. **ISOLATION AND PURIFICATION OF ZOOPLANKTON ASSOCIATED BACTERIA:**

   Zooplankton-associated bacteria were isolated following Wang et al. (2021) with some modifications. 20 liters of seawater samples were filtered through the 200 µm mesh to collect the zooplankton samples. Zooplanktons retained on the mesh were cleaned with the filter autoclaved seawater, collected with a clean spatula and dispensed into the tube containing filtered and autoclaved seawater. The same sample was sonicated and dilutions up to 10^{-5} were prepared and plated on ZMA agar. Plates were incubated at 37^0C for 48 hours. Growth was counted on all the plates. Subsequently, selected bacterial colonies were purified on ZMA plates.

2.BIOCHEMICAL TESTS:

Gram staining and biochemical tests were performed for all the cultures (AL-Joda and Jasim,2021)

3. PROCEDURE FOR RESPIRATION RATES:

Bacterial metabolic rate measurements:

For the said measurements, a 2L estuarine water sample was filtered using 0.22 um polycarbonate membrane filters, followed by autoclaving at 121^0C for 20 minutes. Bacterial respiration (BR) rate was measured in filtered water incubated at room temperature and at 37^0C with the inoculated bacterial cultures as the difference in dissolved oxygen at 0 h and 24 h, 48hrs, 72hrs and so on. Dissolved Oxygen (DO) was analysed using Winkler's titration method of Carritt and Carpenter (1966) by burette. Winkler technique is most commonly used technique to estimate BR, offering high sensitivity (Carignan et al. 1998), although there is a disadvantage of preventing continuous monitoring of oxygen concentrations. Simultaneously filtered water was siphoned out in a 60ml DO bottles (duplicates), wrapped in a black cloth, and incubated at room temperature for checking bacterial abundance (BA). Samples were collected at 0h and 24h from incubated 60 ml DO bottles and preserved at 4 ºC with 5% formalin until analysis.

2 sets were maintained with the selected cultures based on biochemical analysis and enzymatic screening.

Salinity was measured using a salinometer at the start and end of experiment. And periodic amino acid analysis was done by taking OD at 570nm using the Ninhydrin test analysis of the DO bottle at 24hr period (Smith and Agiza,1951)

Protocol for bacterial respiration rate measurements:

Filter 1liter of estuarine water with 0.47 micrometer filter paper

Autoclave at 121^{0}C for 20mins

2 sets of experiment will be maintained along with controls in duplicates

Set 1: Estuarine water+ bacterial culture (at RT and37 ^{0}C)

Set2: distilled water + carbon source (glucose sugar) + bacterial culture (at RT and 37^{0}C)

OD at 600nm will be taken in every two days,

Amino acid measurements will be done using the ninhydrin test

4. PROTOCOL FOR AMINO ACID MEASUREMENTS by Ninhydrin test:

- **For qualitative analysis**
    - Combine 1ml of sample + few drops of ninhydrin reagent in a dry test tube
    - Combine 1ml of standard protein solution +few drops of ninhydrin reagent
    - Heat the tubes for 5mins in water bath, let the tube cool to RT. Look for color change.

- **For quantitative analysis:**
- For plotting a standard curve, first pipette out an increasing volume of protein stock as given in the table. Later make up the volume to 1ml with distilled water.
- Blank was maintained using 1ml distilled water.
- At 48 hours periodic samples were collected from the DO bottles for protein estimation using Ninhydrin solution.
- Finally, 1ml of freshly prepared Ninhydrin solution and 5ml of diluent was added to each tube and mixed by vortex.
- The tubes were then covered and kept in water bath for 20 minutes at 90^0C.
- Tubes were later allowed to cool at room temperature and optical density at 570nm was taken respectively of each sample.
- To quantify the amount of amino acid in the collected samples, the readings were plotted on the graph.

5. TO MEASURE DISSOLVED OXYGEN(DO) BY WINKLER METHOD:

Reagents: $MnSO_4.4H_2O$(2ml), iodide solution (2ml), sodium thiosulphate (0.03N), starch indicator.

Procedure: fill DO bottle with sample, allow it to overflow to avoid any air bubbles in a sample.

1) Add 1 ml of Winkler solution A and 1 ml of Winkler B solution to the filled DO bottle, carefully by inserting the pipette tip below the water surface.
2) Re-stopper and mix by inverting the bottle 10 to 15 times.

3) Allow the bottle to settle for approx. 1 hour so that the brown floc occupies ½ the bottle volume or less.

4) Carefully, add 1ml of 50% H_2SO_4 solution

5) Stopper and shake until the solution, is uniformly mixed.

6) Pour the entire contents into a 500ml Erlenmeyer flask.

7) While swirling the flask, titrate with 0.0375N sodium thiosulfate solution from the starting orange-yellow until it becomes a pale-yellow colour.

8) Add 1ml starch solution, the sample will turn blue.

9) Continue titration until the blue goes to clear.

10) Check the endpoint when the solution is colourless

6. TO CHECK THE ENZYMATIC ACTIVITY

500ml of Zobell marine agar was made according to the composition and autoclaved at 121^0c for 20 minutes. Soluble starch 0.5% was separately autoclaved to check amylase activity, 0.5% of CMC, was also sterilised separately for 10 minutes. Plates for the respective activity was poured using the particular substrate. Later 48 hrs old culture was spot inoculated on the plates and plates were incubated at room temperature for 6 to 7 days.

Amylase activity was checked by flooding the plates with iodine solution

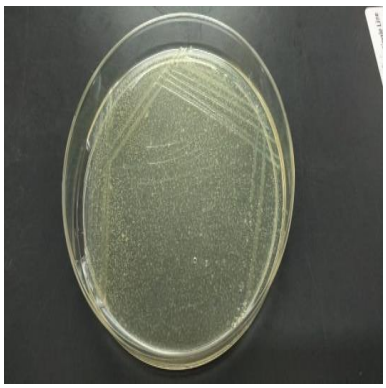
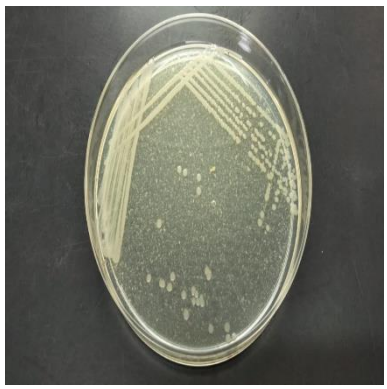
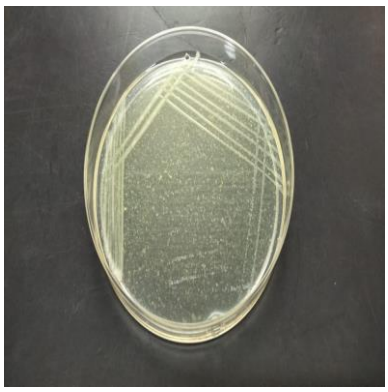
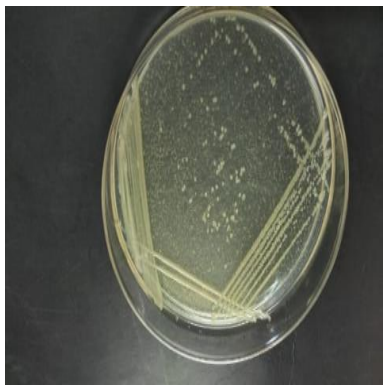
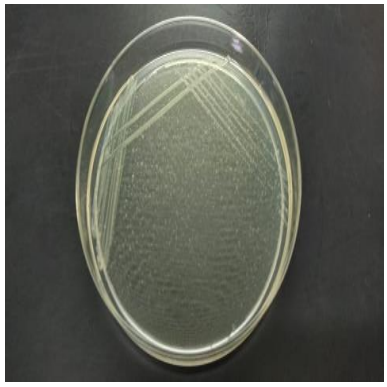
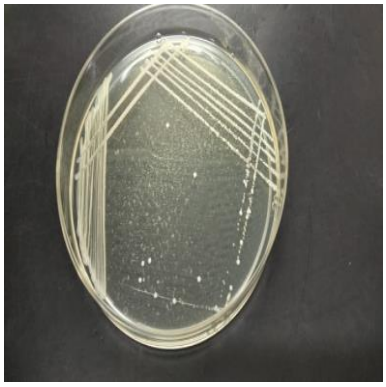
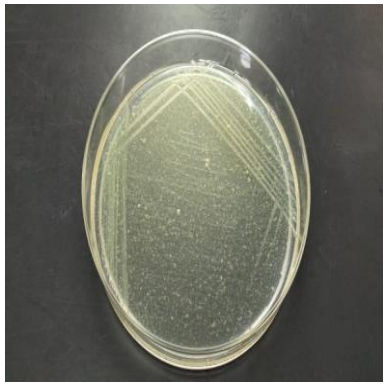
CMC activity was seen by flooding the plates with 0.5% Congo red solution.

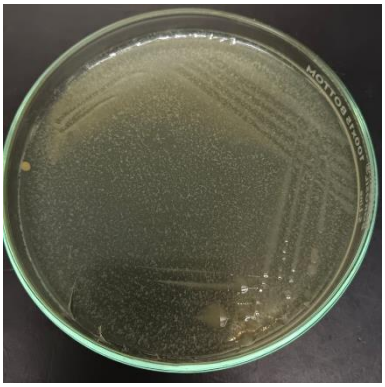
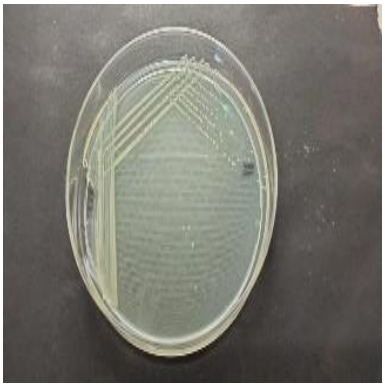
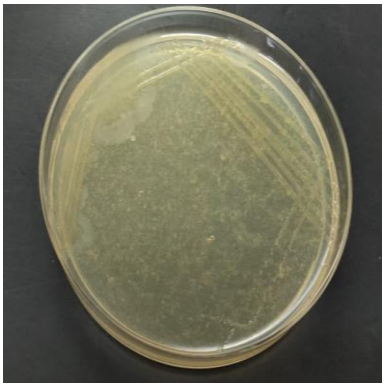
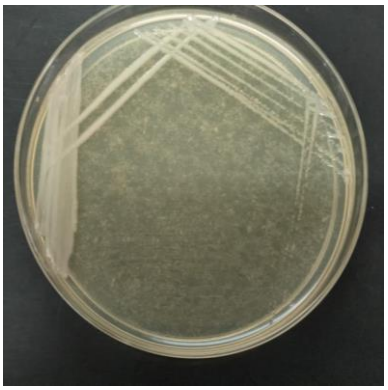
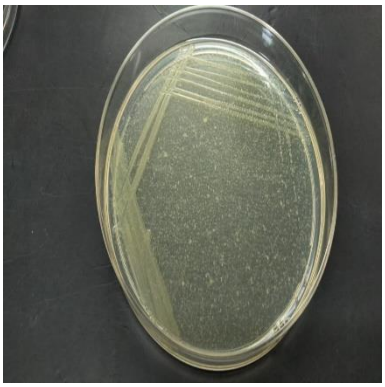
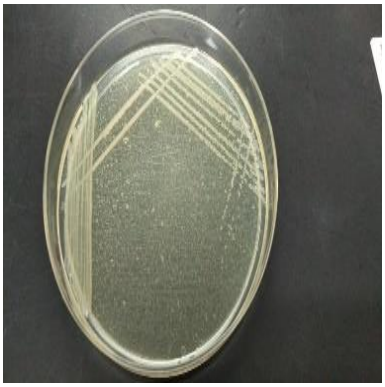
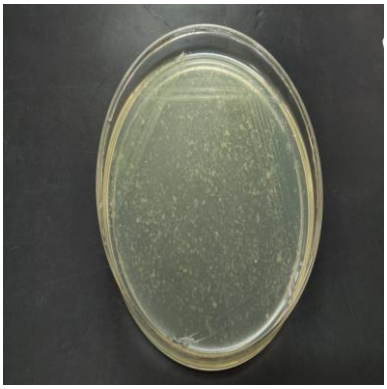
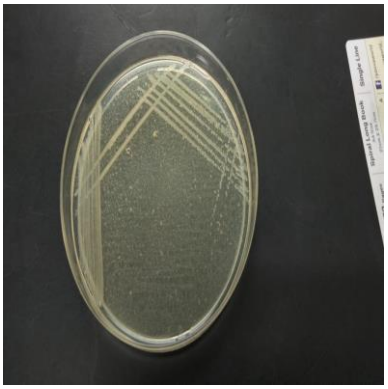
4.RESULTS

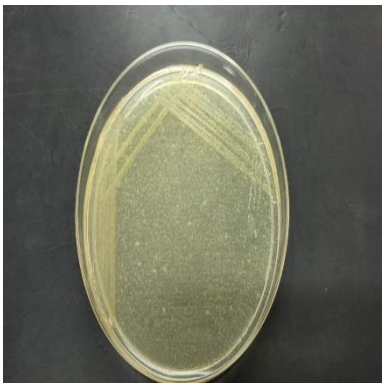
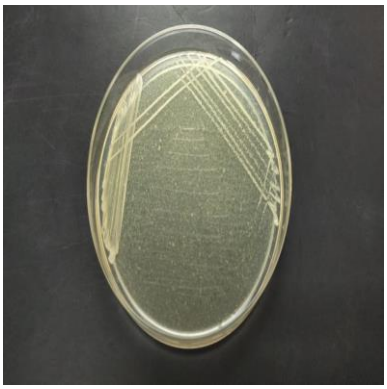
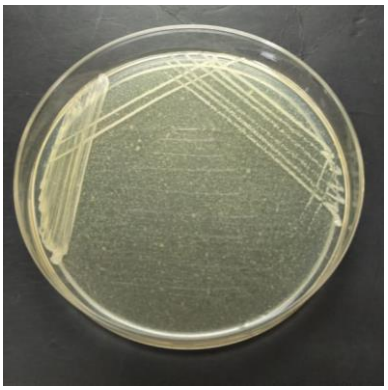
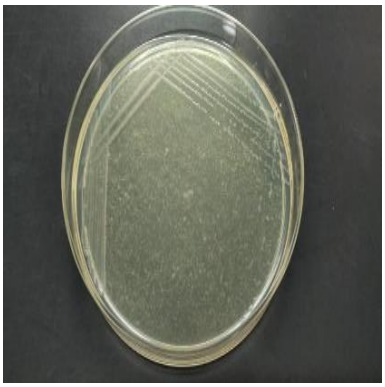
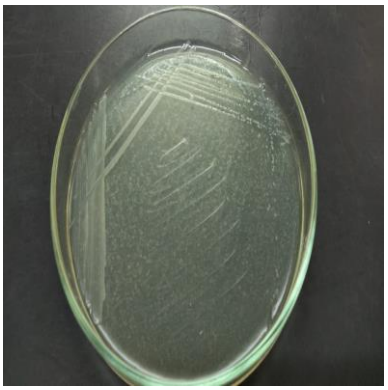
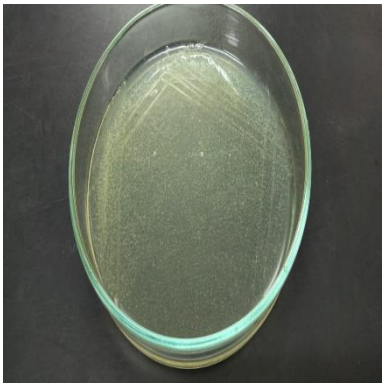
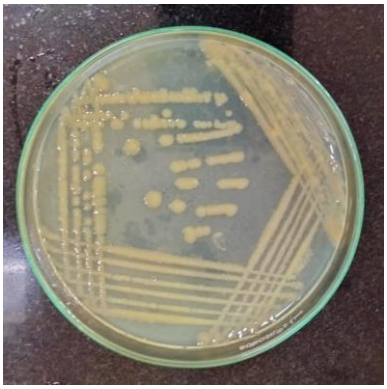
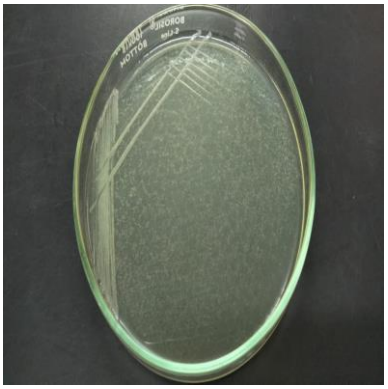
AND

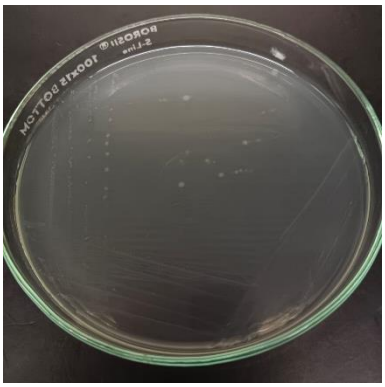
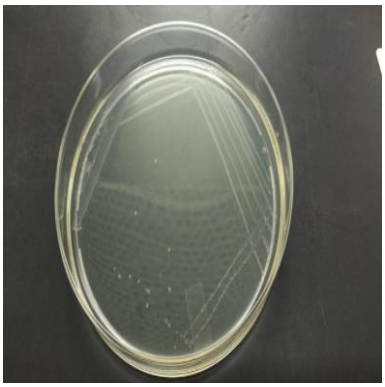
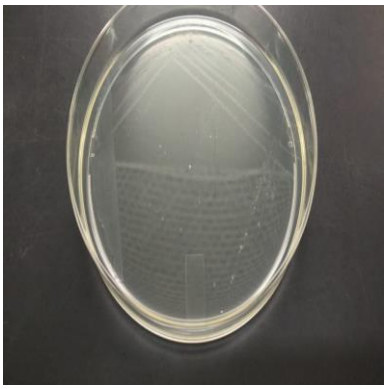
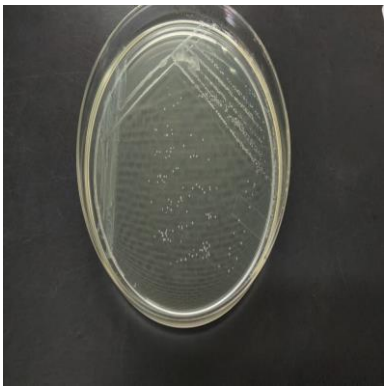
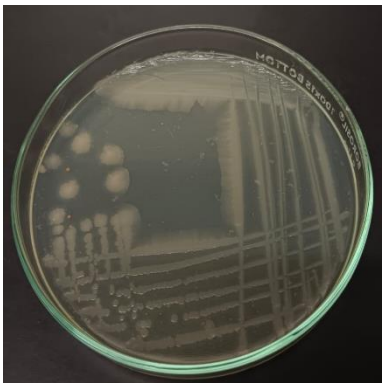
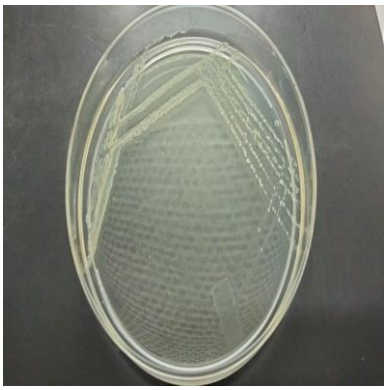
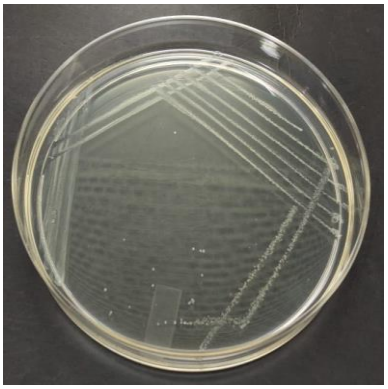
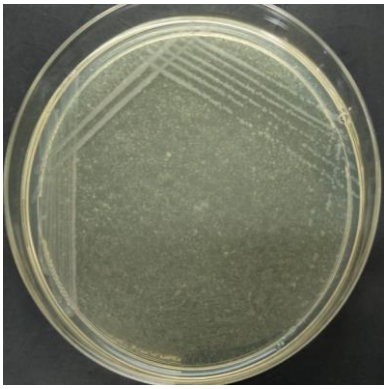
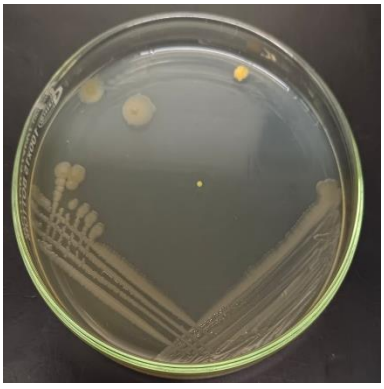
DISCUSSION

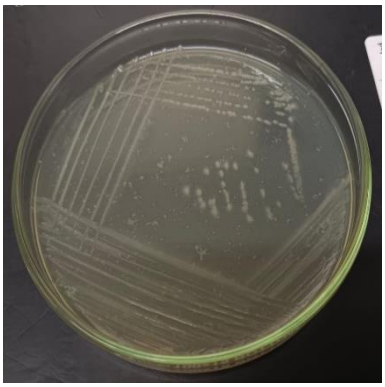
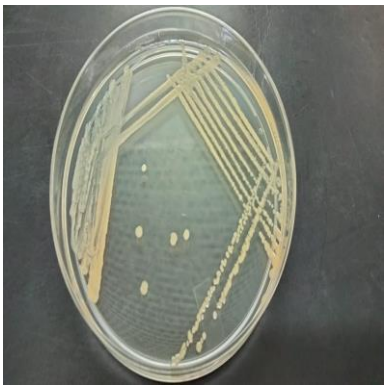
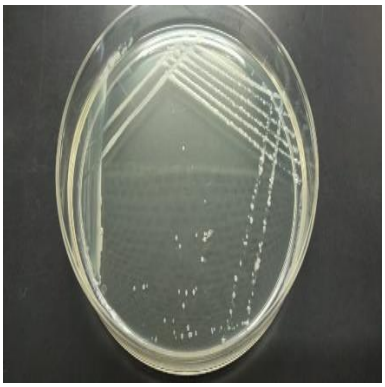
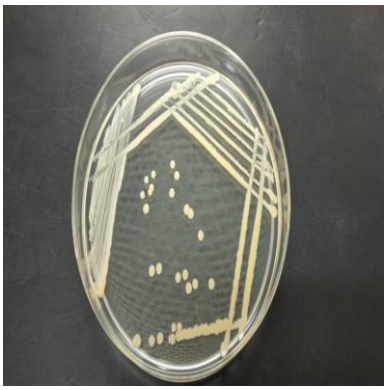
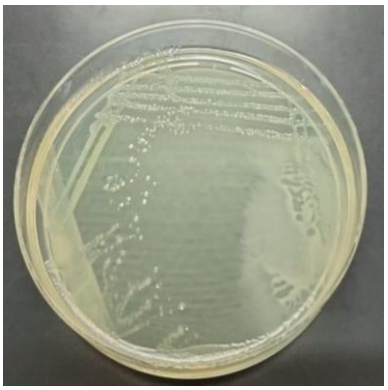
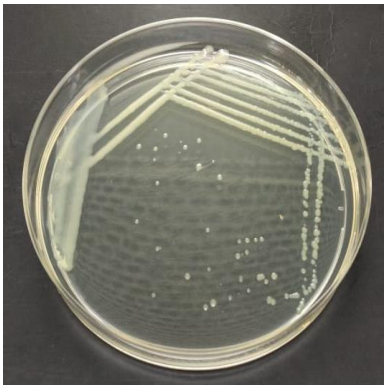
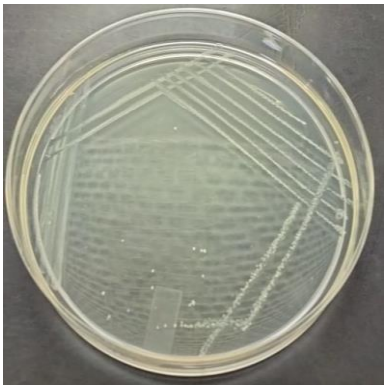
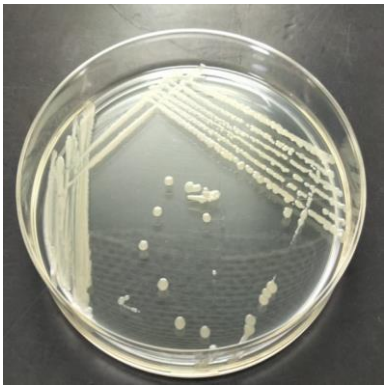
PURIFICATION OF BACTERIAL ISOLATES: TABLE. NO. 4.1

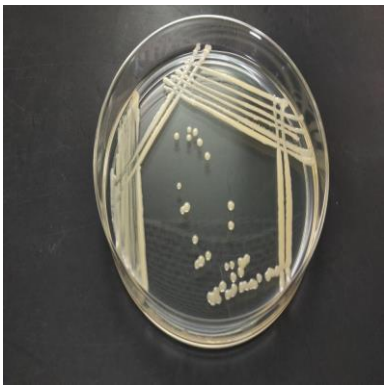
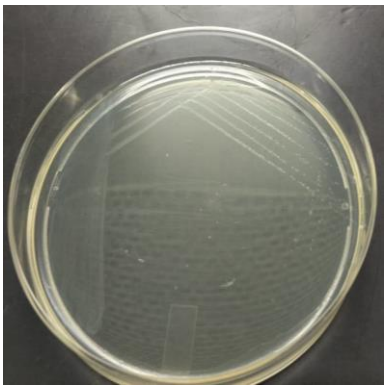
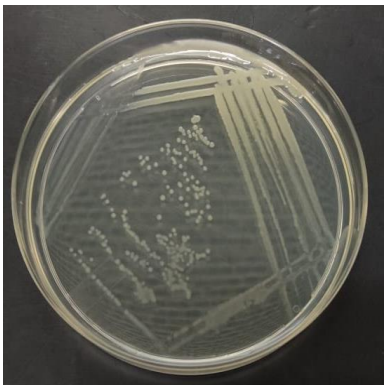
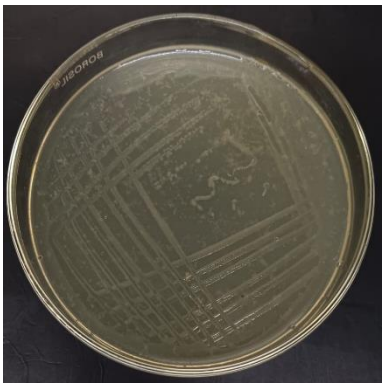
KG3-NF-6T-1Z 	KG3-NF-6T-2Z 	KG3-NF-6T-3Z 
KG3-NF-6T-4Z 	KG3-NF-13T-5Z 	KG3-NF-13T-6Z 
KG3-NF-13T-7Z 	KG3-NF-13T-8Z 	KG3-NF-RT-9Z 

KG3-NF-RT-10Z 	KG3-NF-6T-11Z 	KG3-NF-6T-12Z 
KG3-NF-RT-13Z 	KG3-NF-RT-14Z 	KG3-NF-6T-15Z 
KG3-NF-13T-16Z 	KG3-NF-6T-17Z 	KG3-NF-13T-18Z 

KG3-NF-6T-19Z 	KG3-NF-6T-20Z 	KG3-NF-RT-21Z 
KG3-NF-RT-22Z 	KG3-NF-RT-23Z 	KG3-NF-RT-24Z 
KG3-NF-6T-25Z 	KG3-NF-13T-26Z 	KG3-NF-6T-27Z 

KG3-NF-6T-28Z 	KG3-NF-6T-29Z 	KG3-NF-6T-30Z 
KG3-NF-13T-31Z 	KG3-NF-13T-32Z 	KG3-NF-13T-33Z 
KG3-NF-13T-34Z 	KG3-NF-13T-35Z 	KG3-NF-13T-36Z 

KG3-NF-RT-37Z 	KG3-NF-6T-38Z 	KG3-NF-13T-39Z 
KG3-NF-6T-40Z 	KG4-NF-6T-41Z 	KG3-NF-13T-42Z 
KG3-NF-6T-43Z 	KG3-NF-6T-44Z 	KG3-NF-6T-45Z 

KG3-NF-RT-46Z 	KG3-NF-RT-47Z 	KG3-NF-RT-48Z 
KG3-NF-6T-49Z 	KG3-NF-RT-50Z 	

COLONY CHARACTERIZATION AND BIOCHEMICAL IDENTIFICATION:

TABLE NO. 4.2

Colony characterization:

Colony no.	KG3-NF-6T-1Z	KG3-NF-6T-2Z	KG3-NF-6T-3Z	KG3-NF-6T-4Z	KG3-NF-13T-5Z
MEDIA	ZMA	ZMA	ZMA	ZMA	ZMA
TEMPERATURE	RT	RT	RT	RT	RT
TIME	48hrs	48hrs	48hrs	48hrs	48hrs
SIZE	7mm	5mm	3mm	6mm	7mm
SHAPE	Circular	Circular	Circular	Circular	Irregular
COLOUR	Creamy yellow	Creamy brown	Light pink	Creamy	Creamy
MARGIN	Entire	Undulate	Entire	Curled	Undulate
ELEVATION	Convex	Raised	Raised	Raised	Raised
SURFACE TEXTURE	Smooth	smooth	smooth	Mucoid	Smooth
OPACITY	Opaque	Opaque	Opaque	Opaque	Opaque
GRAM CHARACTER	Gram negative rods	Gram negative short rods	Gram positive rods	Gram negative rods	Gram negative rods

TABLE NO. 4.3

BIOCHEMICAL TESTS:

COLONY NO.	KG3-NF-6T-1Z	KG3-NF-6T-2Z	KG3-NF-6T-3Z	KG3-NF-6T-4Z	KG3-NF-13T-5Z
Indole test	-	-	-	-	-
Methyl red test	-	-	-	-	-
Voges Proskauer test	-	-	-	-	-
Citrate utilisation	-	+	-	-	+
Catalase test	-	+	+	+	+
Urease test	-	-	-	+	-
Motility test	-	-	-	-	-
TSI test a) slant	-	-	-	-	-
b) butt	-	-	-	YELLOW	-
c) H_2S production	-	-	-	-	-
d) Gas production	-	-	-	-	-
Oxidase test	POSITIVE	NEGATIVE	-	-	-
SUGAR FERMENTATION a) glucose	-	-	-	+	+
b) sucrose	-	-	-	+	-
c) lactose	-	-	-	+	-
d) mannitol	-	-	-	+	-
e) maltose	-	-	-	+	+
f) sorbitol	-	-	-	-	-

TABLE NO.4.4

Colony characterization:

Colony no.	KG3-NF-13T-6Z	KG3-NF-13T-7Z	KG3-NF-13T-8Z	KG3-NF-RT-9Z	KG3-NF-RT-10Z
MEDIA	ZMA	ZMA	ZMA	ZMA	ZMA
TEMPERATURE	RT	RT	RT	RT	RT
SIZE	9mm	3mm	23mm	6mm	2mm
SHAPE	Irregular	Irregular	Irregular	Irregular	Circular
COLOUR	Creamy yellow	Creamy	Creamy light yellow	Creamy	Creamy yellow
MARGIN	Undulate	Undulate	Undulate	Undulate	Entire
ELEVATION	Raised	Raised	Flat	Raised	Raised
SURFACE TEXTURE	Smooth	Smooth	Smooth	Smooth	smooth
OPACITY	Opaque	Opaque	Opaque	Opaque	Opaque
GRAM CHARACTER	Gram negative rods	Gram negative short rods	Gram positive cocci	Gram negative short rods	Gram positive cocci

TABLE NO. 4.5

BIOCHRMICAL TESTS:

COLONY NO.	KG3-NF-13T-6Z	KG3-NF-13T-7Z	KG3-NF-13T-8Z	KG3-NF-RT-9Z	KG3-NF-RT-10Z
Indole test	-	-	+	-	-
Methyl red test	-	-	+	-	-
Voges Proskauer test	-	-	-	-	-
Citrate utilisation	+	+	+	+	+
Catalase test	+	+	+	+	+
Urease test	-	-	+	-	-
Motility test	-	-	+	-	-
TSI test a) slant	-	-	+	-	PINK
b) butt	YELLOW	-	+	-	-
c) H_2S production	-	-	+	-	-
d) Gas production	+	-	+	-	-
Oxidase test	POSITIVE	-	-	-	-
SUGAR FERMENTATION a) Glucose	+	+	+	-	+
b) Sucrose	-	-	+	-	-
c) Lactose	-	-	+	-	-
d) Mannitol	-	+	+	-	-
e) Maltose	-	+	+	-	-
f) Sorbitol	-	-	+	-	-

TABLE NO.4. 6

Colony characterization:

Colony no.	KG3-NF-6T-11Z	KG3-NF-6T-12Z	KG3-NF-RT-13Z	KG3-NF-RT-14Z	KG3-NF-6T-15Z
MEDIA	ZMA	ZMA	ZMA	ZMA	ZMA
TEMPERATURE	RT	RT	RT	RT	RT
SIZE	5mm	4mm	10mm	8mm	2mm
SHAPE	Circular	Irregular	Irregular	Irregular	Circular
COLOUR	Creamy	Creamy orange	Creamy	Light orange	Peach
MARGIN	Entire	Undulate	Undulate	Undulate	Entire
ELEVATION	Raised	Raised	Raised	Raised	Convex
SURFACE TEXTURE	Smooth	Smooth	butyrous	butyrous	smooth
OPACITY	Opaque	Opaque	Opaque	Opaque	Opaque
GRAM CHARACTER	Gram positive cocci	Gram negative long rods	Gram negative long rods	Gram positive cocci	Gram positive cocci

TABLE NO. 4.7

BIOCHRMICAL TESTS:

COLONY NO.	KG3-NF-6T-11Z	KG3-NF-6T-12Z	KG3-NF-RT-13Z	KG3-NF-RT-14Z	KG3-NF-6T-15Z
Indole test	-	-	-	-	-
Methyl red test	-	-	-	-	-
Voges Proskauer test	-	-	-	-	-
Citrate utilisation	-	-	+	-	-
Catalase test	-	+	+	+	+
Urease test	-	-	+	-	-
Motility test	-	-	-	-	-
TSI test a) slant	-	-	-	-	-
b) butt	-	-	-	-	-
c) H_2S production	-	-	-	-	-
d) Gas production	-	-	-	-	-
Oxidase test	POSITIVE	POSITIVE	-	-	POSITIVE
SUGAR FERMENTATION a) Glucose	-	-	-	-	-
b) Sucrose	-	-	-	-	-
c) Lactose	-	-	-	-	-
d) Mannitol	-	-	-	-	-
e) Maltose	-	-	-	-	-
f) Sorbitol	-	-	-	-	-

TABLE NO. 4.8

Colony characterization:

Colony no.	KG3-NF-13T-16Z	KG3-NF-6T-17Z	KG3-NF-13T-18Z	KG3-NF-6T-19Z	KG3-NF-6T-20Z
MEDIA	ZMA	ZMA	ZMA	ZMA	ZMA
TEMPERATURE	RT	RT	RT	RT	RT
SIZE	3mm	2mm	5mm	6mm	2mm
SHAPE	Circular	Circular	Irregular	Circular	Circular
COLOUR	Creamy pink	Creamy brown	Creamy brown	Creamy brown	Yellow
MARGIN	Entire	Entire	Undulate	Entire	Entire
ELEVATION	Raised	Umbonate	Umbonate	Convex	Convex
SURFACE TEXTURE	Butyrous	Butyrous	Smooth	Smooth	Smooth
OPACITY	Opaque	Opaque	Opaque	Opaque	Opaque
GRAM CHARACTER	Gram negative short rods	Gram positive cocci	Gram positive cocci	Gram positive cocci	Gram positive cocci

TABLE NO. 4.9

BIOCHRMICAL TESTS:

COLONY NO.	KG3-NF-13T-16Z	KG3-NF-6T-17Z	KG3-NF-13T-18Z	KG3-NF-6T-19Z	KG3-NF-6T-20Z
Indole test	-	-	-	-	-
Methyl red test	-	-	-	-	-
Voges Proskauer test	-	-	-	-	-
Citrate utilisation	+	+	-	-	-
Catalase test	+	+	+	+	+
Urease test	-	+	-	-	+
Motility test	-	-	-	-	-
TSI test a) slant	-	Pink	+	Pink	+
b) butt	-	-	-	-	+
c) H_2S production	-	-	-	-	-
d) Gas production	-	-	-	-	-
Oxidase test	POSITIVE	-	POSITIVE	-	-
SUGAR FERMENTATION a) Glucose	-	+	+	+	+
b) Sucrose	-	-	-	-	-
c) Lactose	-	-	-	-	+
d) Mannitol	-	-	-	-	-
e) Maltose	-	-	-	-	+
f) Sorbitol	-	-	-	-	-

TABLE NO. 4.10

Colony characterization:

Colony no.	KG3-NF-RT-21Z	KG3-NF-RT-22Z	KG3-NF-RT-23Z	KG3-NF-RT-24Z	KG3-NF-6T-25Z
MEDIA	ZMA	ZMA	ZMA	ZMA	ZMA
TEMPERATURE	RT	RT	RT	RT	RT
SIZE	1mm	5mm	4mm	5mm	5mm
SHAPE	Circular	Irregular	Irregular	Irregular	Irregular
COLOUR	Dark orange	Creamy	Light yellow	Creamy	Peach
MARGIN	Entire	Undulate	Undulate	Lobate	Undulate
ELEVATION	Convex	Raised	Flat	Raised	Raised
SURFACE TEXTURE	Mucoid	Smooth	Mucoid	Smooth	Mucoid
OPACITY	Opaque	Opaque	Opaque	Opaque	Opaque
GRAM CHARACTER	Gram negative long rods	Gram negative short rods	Gram negative long rods	Gram negative long rods	Gram negative short rods

TABLE NO. 4.11

BIOCHRMICAL TESTS:

COLONY NO.	KG3-NF-RT-21Z	KG3-NF-RT-22Z	KG3-NF-RT-23Z	KG3-NF-RT-24Z	KG3-NF-6T-25Z
Indole test	-	-	-	-	-
Methyl red test	-	-	-	-	-
Voges Proskauer test	-	-	-	-	-
Citrate utilisation	-	+	-	-	+
Catalase test	+	+	+	+	+
Urease test	-	-	+	+	-
Motility test	-	-	-	-	-
TSI test a) slant	-	+	Yellow	Yellow	+
b) butt	-	-	Yellow	Yellow	-
c) H_2S production	-	-	-	-	-
d) Gas production	-	-	-	-	-
Oxidase test	POSITIVE	-	-	-	-
SUGAR FERMENTATION a) Glucose	-	+	+	+	+
b) Sucrose	-	-	+	-	-
c) Lactose	-	-	-	-	-
d) Mannitol	-	-	-	-	-
e) Maltose	-	+	+	-	-
f) Sorbitol	-	-	-	-	-

TABLE NO.4.12

Colony characterization:

Colony no.	KG3-NF-13T-26Z	KG3-NF-6T-27Z	KG3-NF-6T-28Z	KG3-NF-6T-29Z	KG3-NF-6T-30Z
MEDIA	ZMA	ZMA	ZMA	ZMA	ZMA
TEMPERATURE	RT	RT	RT	RT	RT
SIZE	5mm	2mm	3mm	3mm	4mm
SHAPE	Irregular	Circular	Circular	Circular	Circular
COLOUR	Creamy	Creamy	Creamy yellow	Creamy	Creamy
MARGIN	Lobate	Entire	Entire	Entire	Curled
ELEVATION	Raised	Raised	Raised	Raised	Raised
SURFACE TEXTURE	Smooth	Smooth	Smooth	Mucoid	Mucoid
OPACITY	Opaque	Opaque	Opaque	Opaque	Opaque
GRAM CHARACTER	Gram negative short rods	Gram negative rods	Gram negative short rods	Gram positive cocci	Gram positive cocci

TABLE NO. 4.13

BIOCHRMICAL TESTS:

COLONY NO.	KG3-NF-13T-26Z	KG3-NF-6T-27Z	KG3-NF-6T-28Z	KG3-NF-6T-29Z	KG3-NF-6T-30Z
Indole test	-	-	-	-	-
Methyl red test	-	-	-	-	-
Voges Proskauer test	-	-	-	-	-
Citrate utilisation	-	+	+	-	-
Catalase test	-	+	+	-	-
Urease test	-	-	+	+	+
Motility test	-	-	-	-	-
TSI test a) slant	-	+	Pink	Red	-
b) butt	-	-	-	Yellow	-
c) H_2S production	-	-	-	-	-
d) Gas production	+	-	-	-	-
Oxidase test	POSITIVE	POSITIVE	-	POSITIVE	-
SUGAR FERMENTATION a) Glucose	-	+	+	+	+
b) Sucrose	-	-	-	-	-
c) Lactose	-	-	-	-	-
d) Mannitol	-	-	+	-	-
e) Maltose	-	+	+	-	-
f) Sorbitol	-	-	-	-	-

TABLE NO.4.14

Colony characterization:

Colony no.	KG3-NF-13T-31Z	KG3-NF-13T-32Z	KG3-NF-13T-33Z	KG3-NF-13T-34Z	KG3-NF-13T-35Z
MEDIA	ZMA	ZMA	ZMA	ZMA	ZMA
TEMPERATURE	RT	RT	RT	RT	RT
SIZE	4mm	5mm	1mm	6mm	15mm
SHAPE	Irregular	Circular	Irregular	Circular	Irregular
COLOUR	White	Creamy	Light orange	Orange	Creamy yellow
MARGIN	Undulate	Entire	Undulate	Entire	Entire
ELEVATION	Raised	Raised	Raised	Raised	Flat
SURFACE TEXTURE	Smooth	Butyrous	Smooth	Smooth	Butyrous
OPACITY	Opaque	Opaque	Opaque	Opaque	Opaque
GRAM CHARACTER	Gram negative short rods	Gram positive cocci	Gram negative short rods	Gram negative short rods	Gram negative rods

TABLE NO. 4.15

BIOCHRMICAL TESTS:

COLONY NO.	KG3-NF-13T-31Z	KG3-NF-13T-32Z	KG3-NF-13T-33Z	KG3-NF-13T-34Z	KG3-NF-13T-35Z
Indole test	-	-	-	-	-
Methyl red test	-	-	-	-	-
Voges Proskauer test	-	-	-	-	-
Citrate utilisation	-	-	-	+	-
Catalase test	+	+	+	+	+
Urease test	+	-	-	-	+
Motility test	-	-	-	-	-
TSI test a) slant	Yellow	Pink	Pink	Pink	-
b) butt	-	Pink	Pink	Pink	-
c) H_2S production	-	-	-	-	-
d) Gas production	-	-	-	-	-
Oxidase test	-	-	-	-	-
SUGAR FERMENTATION a) Glucose	+	+	+	+	-
b) Sucrose	-	-	-	-	-
c) Lactose	-	-	-	-	-
d) Mannitol	+	-	-	-	-
e) Maltose	+	-	-	+	-
f) Sorbitol	-	-	-	-	-

TABLE NO. 4.16

Colony characterization:

Colony no.	KG3-NF-13T-36Z	KG3-NF-RT-37Z	KG3-NF-6T-38Z	KG3-NF-13T-39Z	KG3-NF-6T-40Z
MEDIA	ZMA	ZMA	ZMA	ZMA	ZMA
TEMPERATURE	RT	RT	RT	RT	RT
SIZE	10mm	7mm	3mm	2mm	3mm
SHAPE	Irregular	Irregular	Irregular	Circular	Circular
COLOUR	Creamy	Peach	White	Creamy	Creamy
MARGIN	Lobate	Undulate	Lobate	Entire	Entire
ELEVATION	Raised	Raised	Flat	Flat	Raised
SURFACE TEXTURE	Smooth	Smooth	Smooth	Smooth	Smooth
OPACITY	Opaque	Opaque	Opaque	Opaque	Opaque
GRAM CHARACTER	Gram positive cocci	Gram negative short rods	Gram negative cocci	Gram negative rods	Gram positive cocci

TABLE NO. 4.17

BIOCHRMICAL TESTS:

COLONY NO.	KG3-NF-13T-36Z	KG3-NF-RT-37Z	KG3-NF-6T-38Z	KG3-NF-13T-39Z	KG3-NF-6T-40Z
Indole test	-	-	-	-	-
Methyl red test	-	-	-	-	-
Voges Proskauer test	-	-	-	-	-
Citrate utilisation	-	+	+	+	-
Catalase test	+	+	+	+	-
Urease test	-	+	+	+	+
Motility test	-	-	-	-	-
TSI test a) slant	Pink	Yellow	Pink	Pink	+
b) butt	Pink	Yellow	Pink	Pink	-
c) H_2S production	-	-	-	-	-
d) Gas production	-	-	-	-	-
Oxidase test	-	-	-	POSITIVE	-
SUGAR FERENTATION a) Glucose	+	+	+	-	+
b) Sucrose	-	+	-	-	-
c) Lactose	-	-	-	-	-
d) Mannitol	-	-	-	-	-
e) Maltose	-	-	-	-	+
f) Sorbitol	-	-	-	-	-

TABLE NO. 4.18

Colony characterization:

Colony no.	KG3-NF-6T-41Z	KG3-NF-13T-42Z	KG3-NF-6T-43Z	KG3-NF-6T-44Z	KG3-NF-6T-45Z
MEDIA	ZMA	ZMA	ZMA	ZMA	ZMA
TEMPERATURE	RT	RT	RT	RT	RT
SIZE	3mm	10mm	2mm	3mm	4mm
SHAPE	Circular	Circular	Circular	Circular	Circular
COLOUR	Creamy yellow	Orange	Pink	Creamy	Creamy pink
MARGIN	Entire	Entire	Entire	Entire	Entire
ELEVATION	Raised	Raised	Convex	Raised	Raised
SURFACE TEXTURE	Smooth	Smooth	Smooth	Smooth	Smooth
OPACITY	Opaque	Opaque	Opaque	Opaque	Opaque
GRAM CHARACTER	Gram positive cocci	Gram negative long rods	Gram negative short rods	Gram positive cocci	Gram positive cocci

TABLE NO.4.19

BIOCHRMICAL TESTS:

COLONY NO.	KG3-NF-6T-41Z	KG3-NF-13T-42Z	KG3-NF-6T-43Z	KG3-NF-6T-44Z	KG3-NF-6T-45Z
Indole test	-	-	-	-	-
Methyl red test	-	-	-	-	-
Voges Proskauer test	-	-	-	-	-
Citrate utilisation	-	+	-	-	-
Catalase test	+	+	+	-	-
Urease test	-	+	-	-	-
Motility test	-	-	-	-	-
TSI test a) slant	Yellow	Pink	-	-	Yellow
b) butt	Yellow	Pink	-	-	Yellow
c) H_2S production	-	-	-	-	-
d) Gas production	-	-	-	-	-
Oxidase test	-	-	POSITIVE	-	-
SUGAR FERMENTATION a) Glucose	+	+	-	-	+
b) Sucrose	+	-	-	-	+
c) Lactose	+	-	-	-	+
d) Mannitol	+	-	-	-	+
e) Maltose	+	-	-	-	+
f) Sorbitol	+	-	-	-	+

TABLE NO.4.20

Colony characterization:

Colony no.	KG3-NF-RT-46Z	KG3-NF-RT-47Z	KG3-NF-RT-48Z	KG3-NF-6T-49Z	KG3-NF-RT-50Z
MEDIA	ZMA	ZMA	ZMA	ZMA	ZMA
TEMPERATURE	RT	RT	RT	RT	RT
SIZE	2mm	3mm	6mm	3mm	10mm
SHAPE	Circular	Circular	Circular	Circular	Irregular
COLOUR	Creamy yellow	Creamy	Creamy brown	Creamy	Yellow
MARGIN	Entire	Entire	Entire	Entire	Undulate
ELEVATION	Raised	Raised	Raised	Convex	Raised
SURFACE TEXTURE	Smooth	Smooth	Smooth	Mucoid	Smooth
OPACITY	Opaque	Opaque	Opaque	Opaque	Opaque
GRAM CHARACTER	Gram negative cocci	Gram negative short rods	Gram negative rods	Gram negative short rods	Gram negative cocci

TABLE NO.4. 21

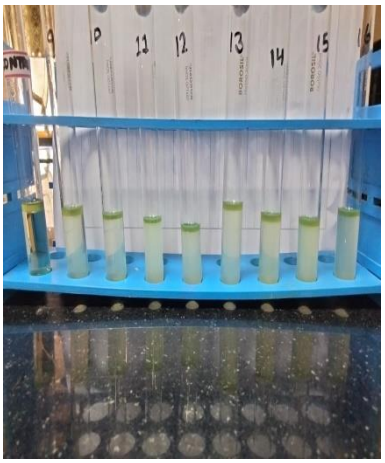
BIOCHRMICAL TESTS:

COLONY NO.	KG3-NF-RT-46Z	KG3-NF-RT-47Z	KG3-NF-RT-48Z	KG3-NF-6T-49Z	KG3-NF-RT-50Z
Indole test	-	-	-	-	-
Methyl red test	-	-	-	-	-
Voges Proskauer test	-	-	-	-	-
Citrate utilisation	-	-	+	+	-
Catalase test	+	+	+	+	+
Urease test	-	+	+	+	-
Motility test	-	-	-	-	-
TSI test a) slant	Yellow	-	Yellow	Pink	Pink
b) butt	Yellow	-	Yellow	-	-
c) H_2S production	-	-	-	-	-
d) Gas production	-	-	-	-	-
Oxidase test	-	-	-	-	-
SUGAR FERMENTATION a) Glucose	+	-	+	+	+
b) Sucrose	-	-	-	-	-
c) Lactose	-	-	-	-	-
d) Mannitol	+	-	+	-	-
e) Maltose	+	-	+	-	-
f) sorbitol	+	-	-	-	-

TABLE NO. 4.22: PICTURES OF BIOCHEMICAL TESTS

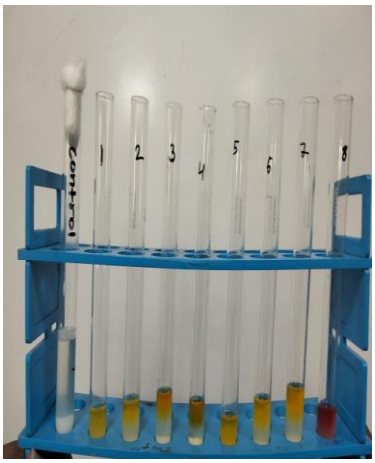
BIOCHEMICAL TESTS:

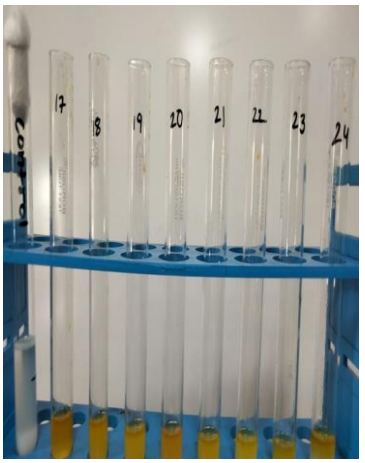
INDOLE TEST

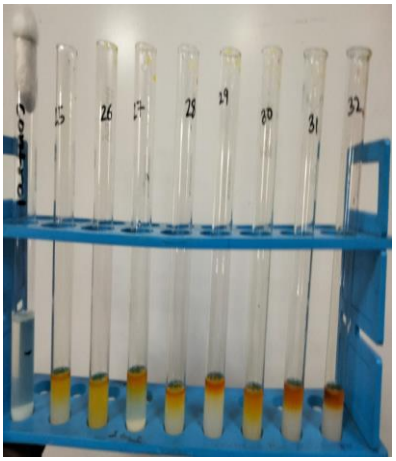





METHYL RED TEST:



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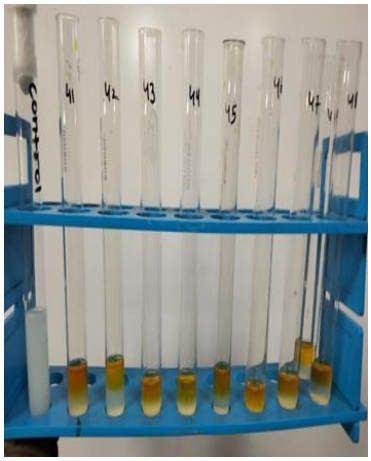
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VP TEST:


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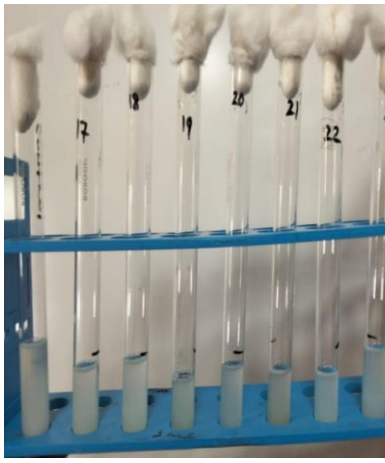
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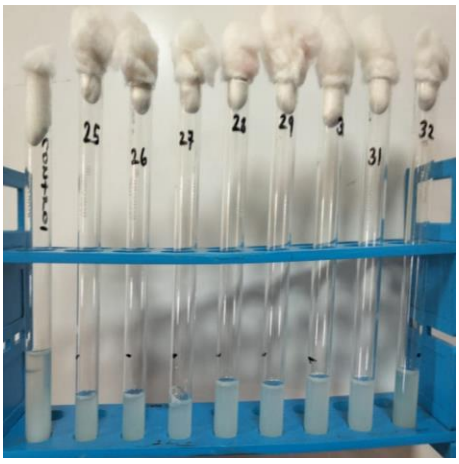
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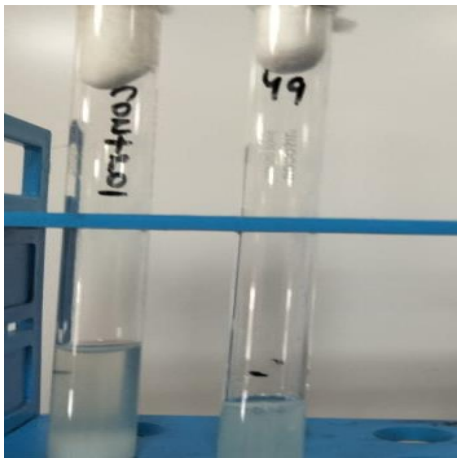
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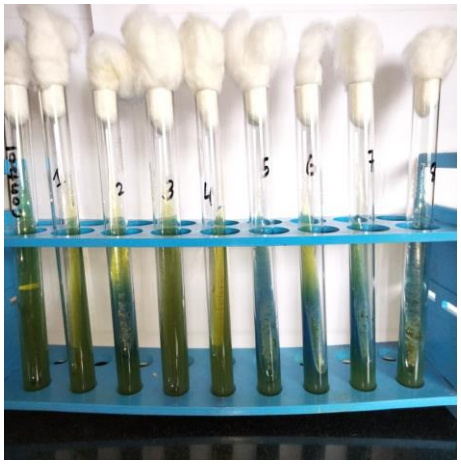
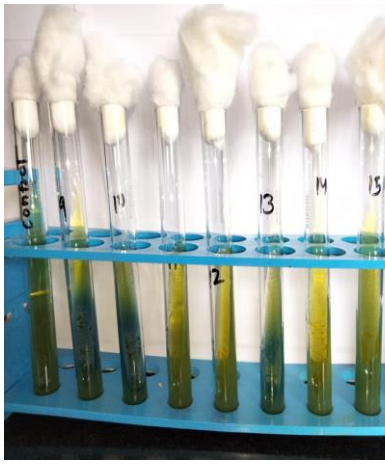
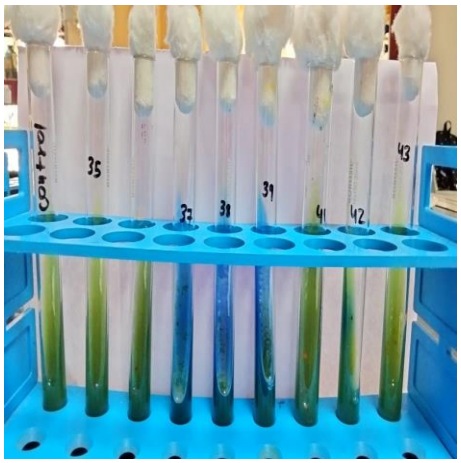
CITRATE TEST







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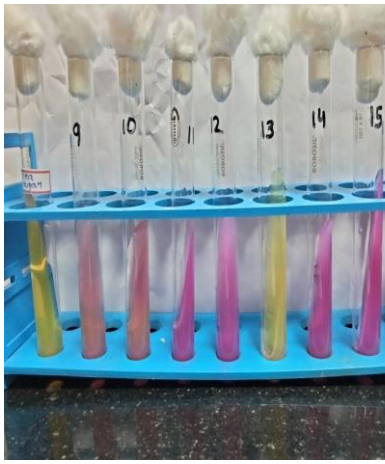

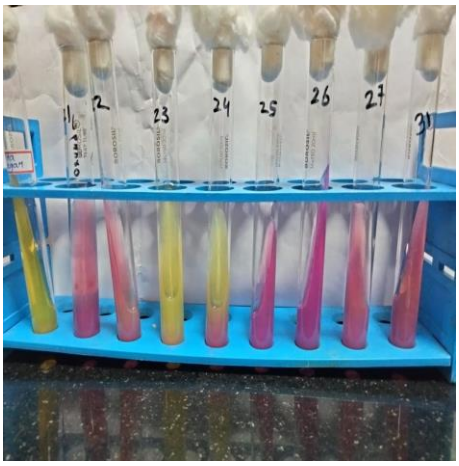

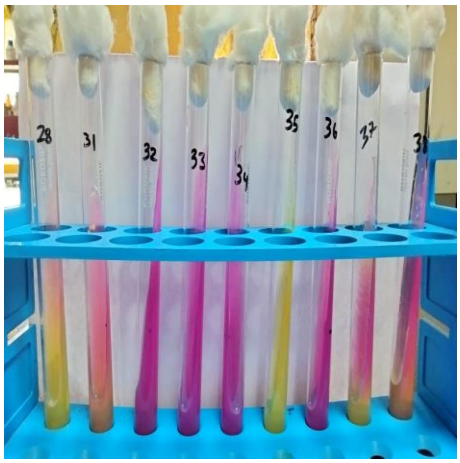

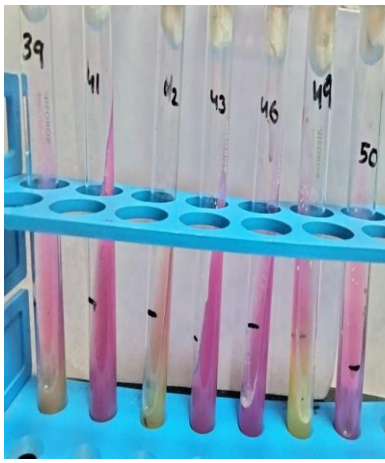

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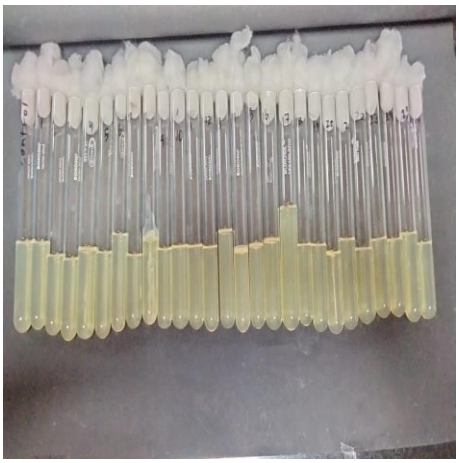
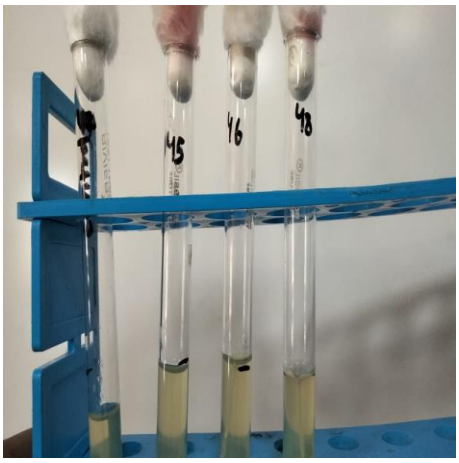
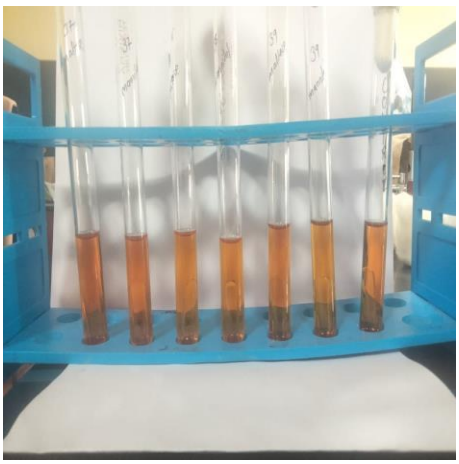
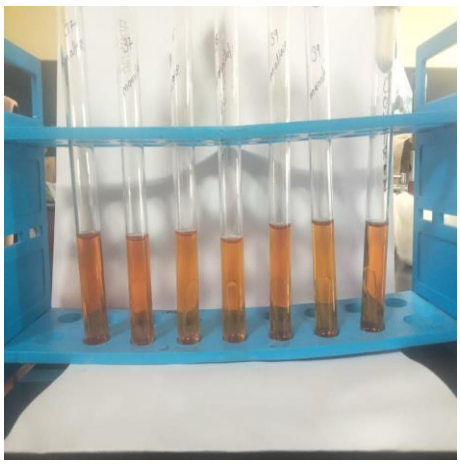


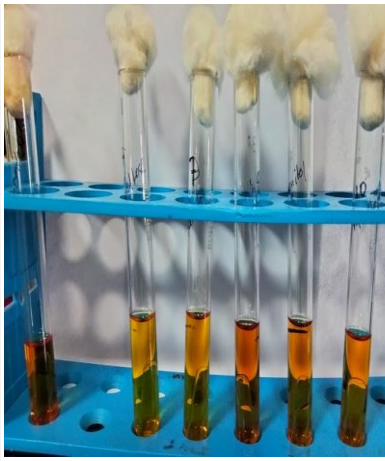
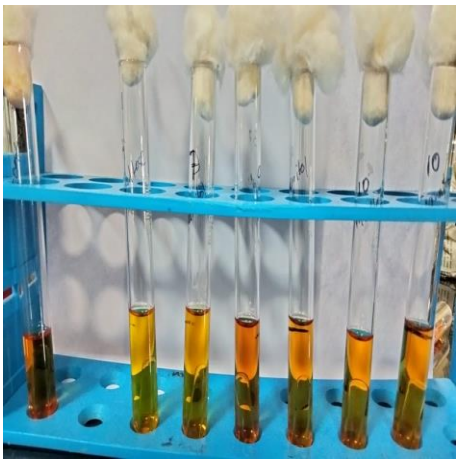
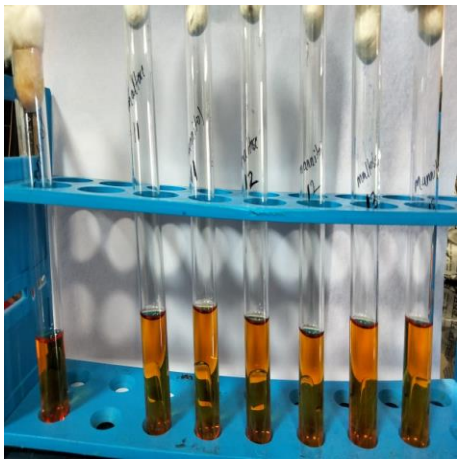


SUGAR TEST



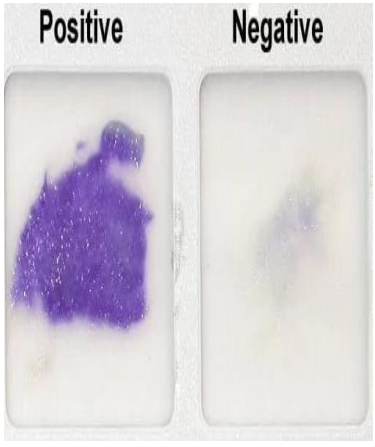




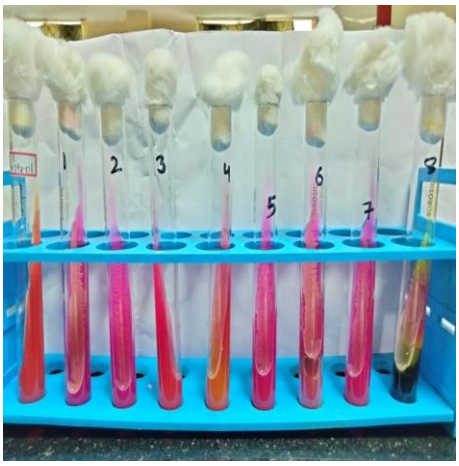





CATALASE TEST:

OXIDASE TEST:

TSI TEST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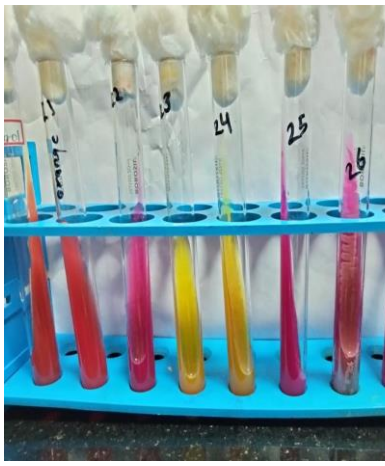
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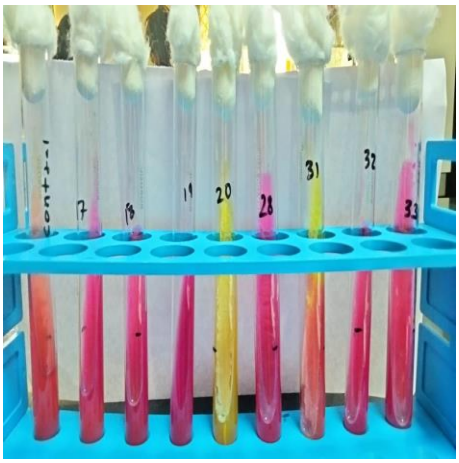
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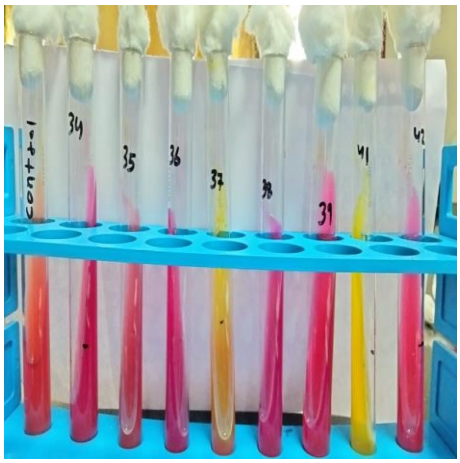
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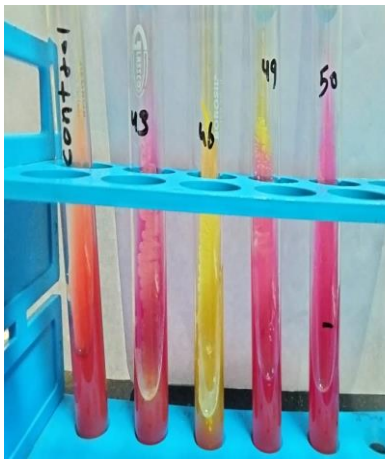
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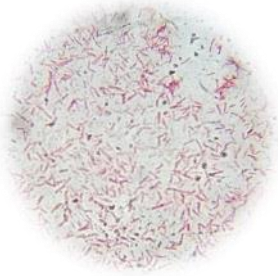
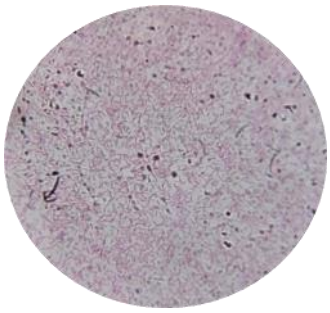
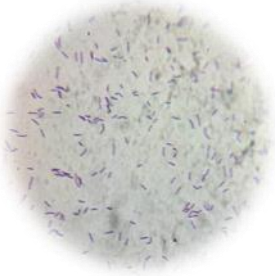
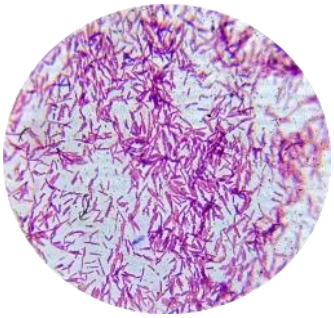
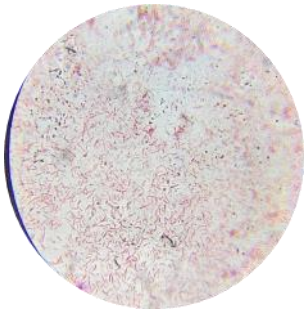
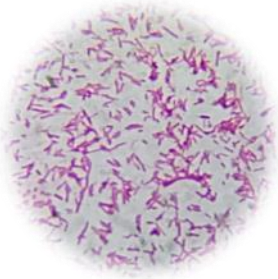
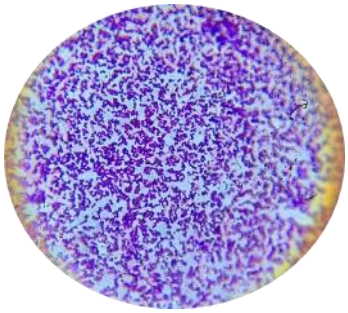
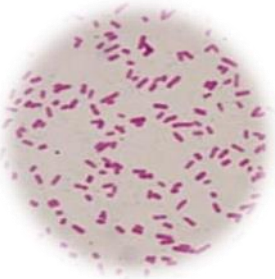
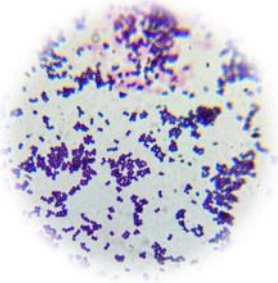
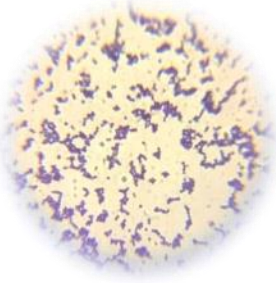
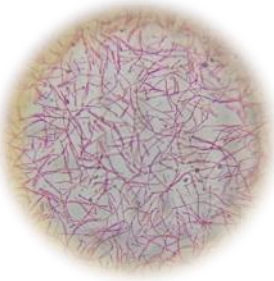
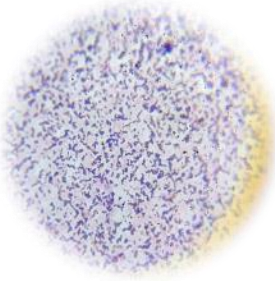
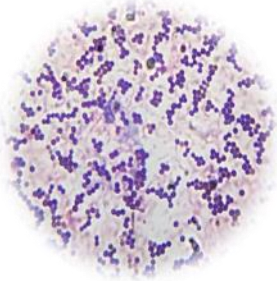
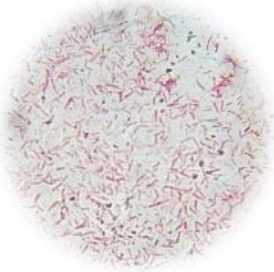
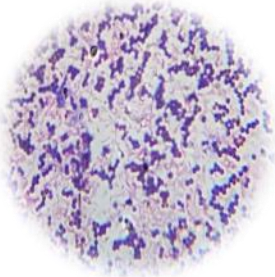
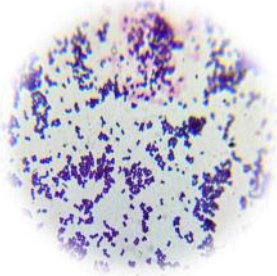
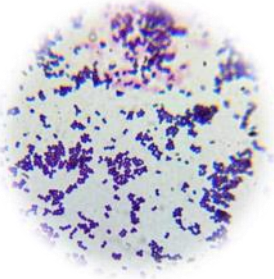
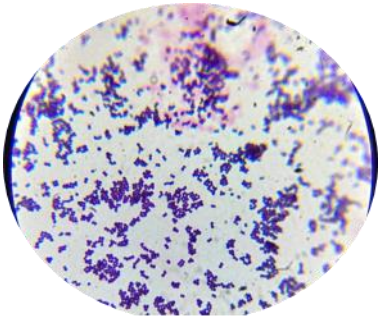
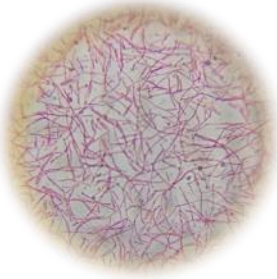
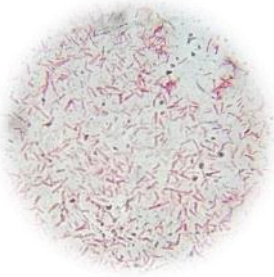
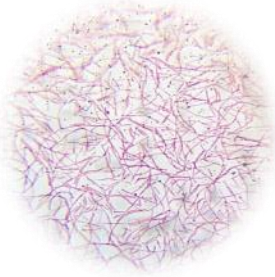
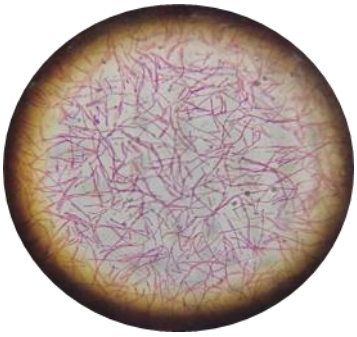
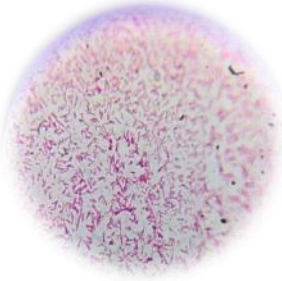
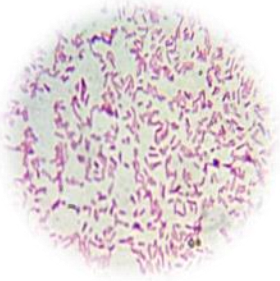
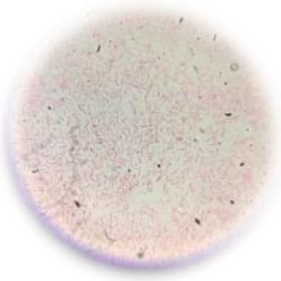
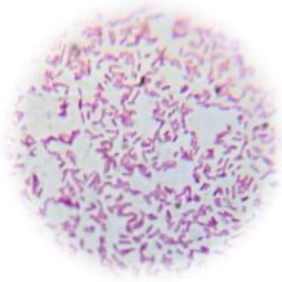
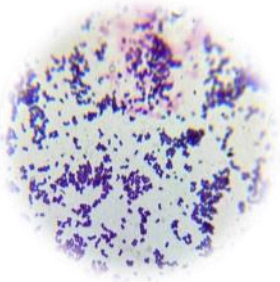
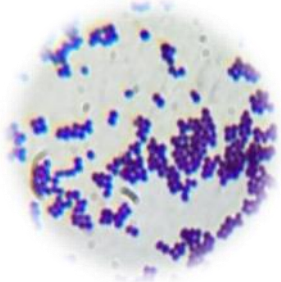
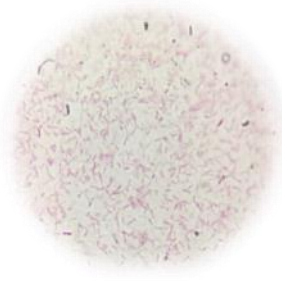
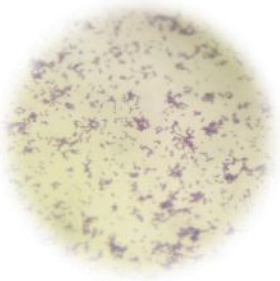
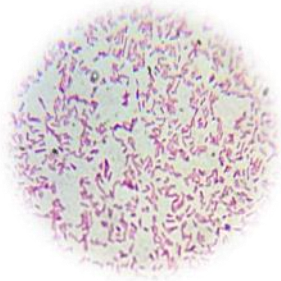
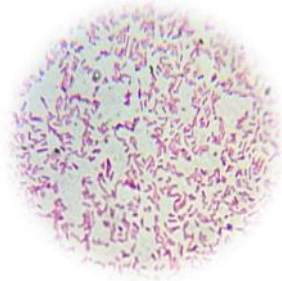
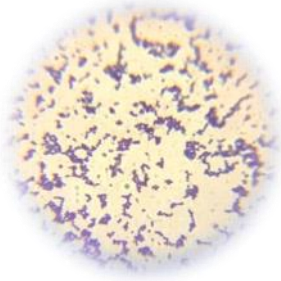
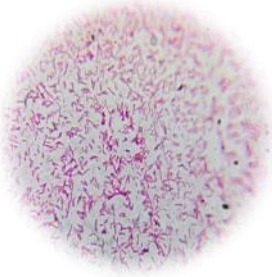
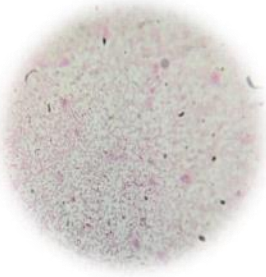
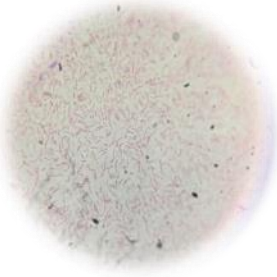
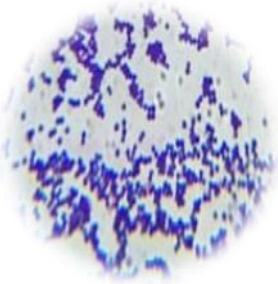
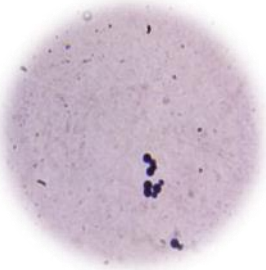
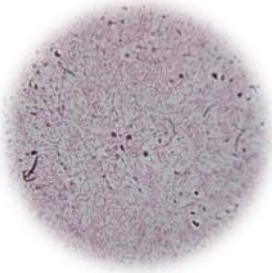
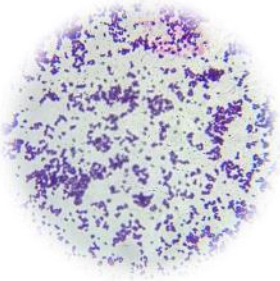
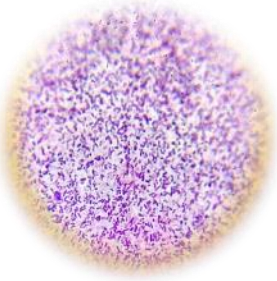
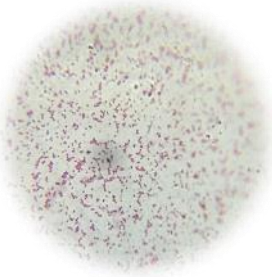
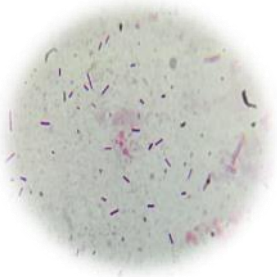
50

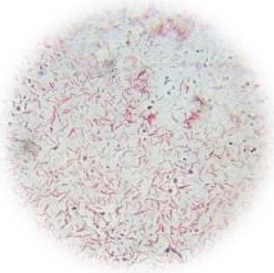
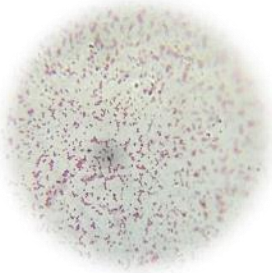
GRAM CHARACTERS OF ALL THE ISOLATES: TABLE NO.4.23

KG3-NF-6T-1Z 	KG3-NF-6T-2Z 	KG3-NF-6T-3Z 
KG3-NF-6T-4Z 	KG3-NF-13T-5Z 	KG3-NF-13T-6Z 
KG3-NF-13T-7Z 	KG3-NF-13T-8Z 	KG3-NF-RT-9Z 
KG3-NF-RT-10Z 	KG3-NF-6T-11Z 	KG3-NF-6T-12Z 

KG3-NF-RT-13Z 	KG3-NF-RT-14Z 	KG3-NF-6T-15Z 
KG3-NF-13T-16Z 	KG3-NF-6T-17Z 	KG3-NF-13T-18Z 
KG3-NF-6T-19Z 	KG3-NF-6T-20Z 	KG3-NF-RT-21Z 
KG3-NF-RT-22Z 	KG3-NF-RT-23Z 	KG3-NF-RT-24Z 

KG3-NF-6T-25Z 	KG3-NF-13T-26Z 	KG3-NF-6T-27Z 
KG3-NF-6T-28Z 	KG3-NF-6T-29Z 	KG3-NF-6T-30Z 
KG3-NF-13T-31Z 	KG3-NF-13T-32Z 	KG3-NF-13T-33Z 
KG3-NF-13T-34Z 	KG3-NF-13T-35Z 	KG3-NF-13T-36Z 

KG3-NF-RT-37Z 	KG3-NF-6T-38Z 	KG3-NF-13T-39Z 
KG3-NF-6T-40Z 	KG3-NF-6T-41Z 	KG3-NF-13T-42Z 
KG3-NF-6T-43Z 	KG3-NF-6T-44Z 	KG3-NF-6T-45Z 
KG3-NF-RT-46Z 	KG3-NF-RT-47Z 	KG3-NF-RT-48Z 

KG3-NF-6T-49Z 	KG3-NF-RT-50Z 	

SCREENING OF ENZYMES

AMYLASE ACTIVITY: Fig. no.4.2

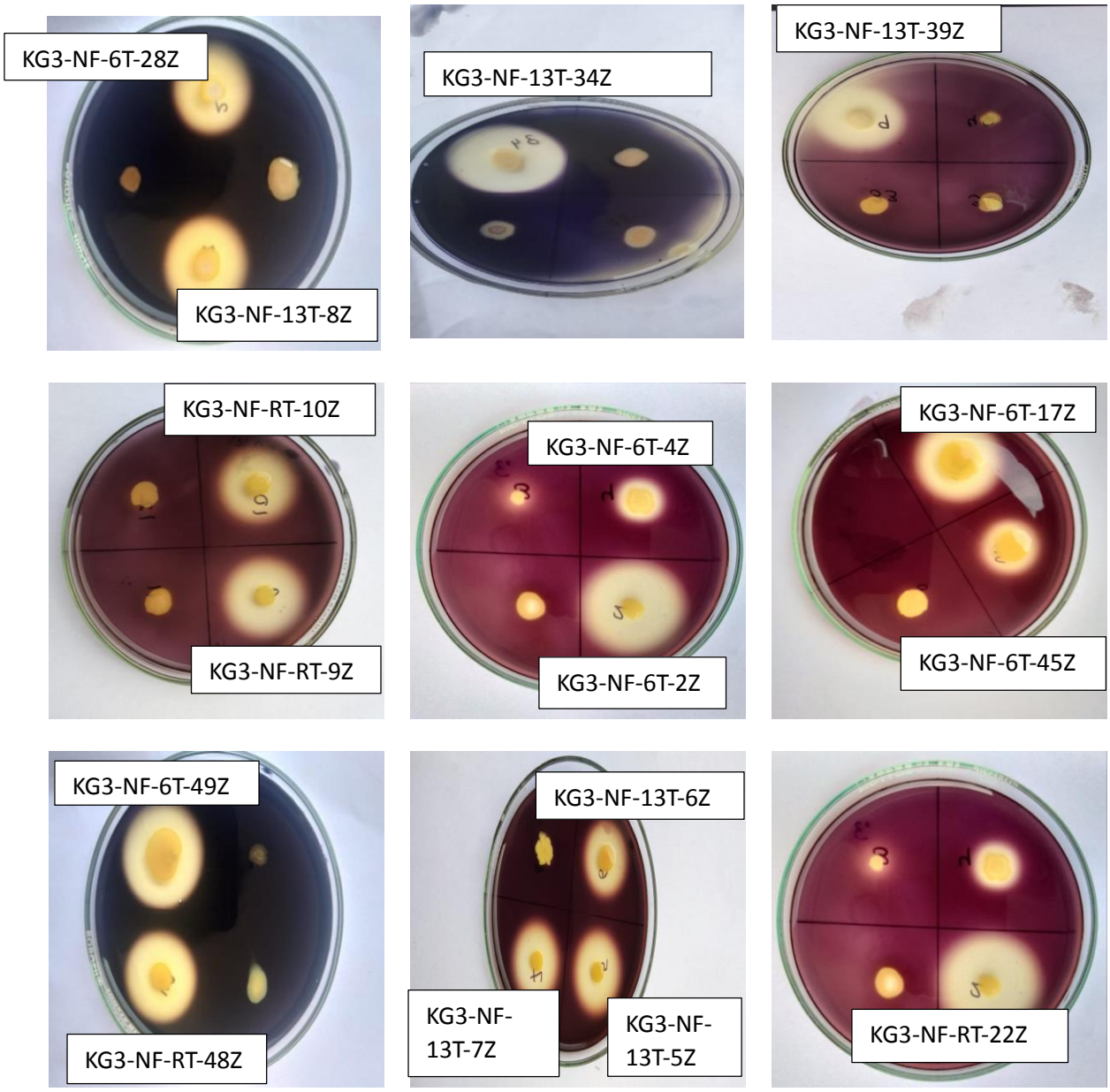


TABLE NO.4.24: AMYLASE ACTIVITY

Culture no.	1	2	3	4	5	6	7	8	9	10
AMYLASE ACTIVITY	-	+	-	+	+	+	+	+	+	+
ZONE SIZE (in cm)	-	3.2 cm	-	1.4 cm	2.8 cm	2.7 cm	2.5 cm	3 cm	2.9 Cm	2.8 Cm
Culture no.	11	12	13	14	15	16	17	18	19	20
AMYLASE ACTIVITY	-	-	-	-	-	-	+	-	-	-
ZONE SIZE (in cm)	-	-	-	-	-	-	3.2 cm	-	-	-
Culture no.	21	22	23	24	25	26	27	28	29	30
AMYLASE ACTIVITY	-	+	-	-	+	-	+	+	-	-
ZONE SIZE (in cm)	-	3 cm	-	-	2.7 cm	-	2.7 cm	2.9 cm	-	-
Culture no.	31	32	33	34	35	36	37	38	39	40
AMYLASE ACTIVITY	-	-	-	+	-	-	-	-	+	-
ZONE SIZE (in cm)	-	-	-	3.5 cm	-	-	-	-	3 Cm	-
Culture no.	41	42	43	44	45	46	47	48	49	50
AMYLASE ACTIVITY	-	-	-	-	+	-	-	+	+	-
ZONE SIZE (in cm)	-	-	-	-	1.8 cm	-	-	3.5 cm	3 Cm	-

TABLE NO. 4.25: CELLULASE ENZYME

CELLULASE ACTIVITY:

Culture no.	1	2	3	4	5	6	7	8	9	10
CELLULASE ACTIVITY	-	-	-	-	-	-	-	-	-	-
Culture no.	11	12	13	14	15	16	17	18	19	20
CELLULASE ACTIVITY	-	-	-	-	-	-	-	-	-	-
Culture no.	21	22	23	24	25	26	27	28	29	30
CELLULASE ACTIVITY	-	-	-	-	-	-	-	-	-	-
Culture no.	31	32	33	34	35	36	37	38	39	40
CELLULASE ACTIVITY	-	-	-	-	-	-	-	-	-	-
Culture no.	41	42	43	44	45	46	47	48	49	50
CELLULASE ACTIVITY	-	-	-	-	-	-	-	-	-	-

METABOLIC RATES

Salinity at the initial: 30%, Salinity at final:5%

BY WINKLER TITRATION:

Observations:

Burette: 0.0375 N $Na_2S_2O_3$

Flask: 60ml of sample + 1ml $MnSO_4$ + 1ml Alkali + indicator

Indicator: 1% starch solution

Colour change: blue black to colourless

CALCULATIONS:

Dissolved oxygen (DO): $N_{Na2S2O3}$*$V_{Na2S2O3}$*8000 / Volume of sample taken

N = normality of Sodium thiosulphate

V = volume of Sodium thiosulphate

OBSERVATION TABLE: AT RT:

TABLE NO. 4.26

Culture no.KG3-NF-13T-8Z (with natural organic carbon source)

BURETTE READING	DAY 1(0HRS)	DAY 2 (24HRS)	DAY 3 (48 HRS)	DAY 4(72HRS)	DAY5(96 HRS)	DAY 6(120HRS)
INITIAL	0	0	0	0	0	0
FINAL	1.9ml	1.8	1.6	0.6	0.2	No change
DIFFERENCE	1.9ml	1.8	1.6	0.6	0.2	No change
AMOUNT OF DO mg/L	9.5	9	8	3	1	0

TABLE NO.4.27

Culture no.KG3-NF-13T-8Z (with Artificial carbon source i.e. Glucose)

BURETTE READING	DAY 1(0HRS)	DAY 2 (24HRS)	DAY 3 (48 HRS)	DAY 4(72HRS)	DAY5(96 HRS)	DAY 6(120HRS)
INITIAL	0	0	0	0	0	0
FINAL	1.2	1.2	0.8	0.6	0.1	No chnage
DIFFERENCE	1.2	1.2	0.8	0.6	0.1	No change
AMOUNT OF DO mg/L	6	6	4	3	0.5	0

TABLE NO. 4.28

Culture no.KG3-NF-6T-48Z (with natural organic carbon source)

BURETTE READING	DAY 1(0HRS)	DAY 2 (24HRS)	DAY 3(48 HRS)	DAY 4 (72 HRS)	DAY 5 (96HRS)	DAY6 (120 HRS)	DAY 7 (144HRS)	DAY 8(168 HRS)
INITIAL	0	0	0	0	0	0	0	0
FINAL	1.9	1.9	1	0.8	0.5	0.2	0.1	No change
DIFFERENCE	1.9	1.9	1	0.8	0.5	0.2	0.1	No change
AMOUNT OF DO mg/L	9.5	9.5	5	4	2.5	1	0.5	0

TABLE NO. 4.29

Culture no.KG3-NF-6T-48Z (with artificial carbon source i.e. Glucose)

BURETTE READING	DAY 1(0HRS)	DAY 2 (24 HRS)	DAY 3 (48 HRS)	DAY 4(72HRS)	DAY5(96 HRS)	DAY 6(120HRS)
INITIAL	0	0	0	0	0	0
FINAL	1.2	1	0.7	0.4	No change	No change
DIFFERENCE	1.2	1	0.7	0.4	No change	No change
AMOUNT OF DO mg/L	6	5	3.5	2	0	0

OBSERVATIONS AT 37^0C:

TABLE NO. 4.30

Culture no.KG3-NF-13T-8Z (with natural organic carbon source)

BURETTE READING	DAY 1(0HRS)	DAY 2 (24HRS)	DAY 3 (48HRS)	DAY 4(72HRS)	DAY5(96 HRS)	DAY 6(120HRS)	DAY 7(144 HRS)	DAY 8(168 HRS)
INITIAL	0	0	0	0	0	0	0	0
FINAL	1.9	1.6	1.3	1	0.5	0.3	0.1	No change
DIFFERENCE	1.9	1.6	1.3	1	0.5	0.3	0.1	No change
AMOUNT OF DO mg/L	9.5	8	6.5	5	2.5	1.5	0.5	0

TABLE NO. 4.31

Culture no.KG3-NF-13T-8Z (with artificial carbon source i.e. Glucose)

BURETTE READING	DAY 1(0HRS)	DAY 2 (24HRS)	DAY 3 (48 HRS)	DAY 4(72HRS)	DAY5(96 HRS)	DAY 6(120HRS)
INITIAL	0	0	0	0	0	0
FINAL	1.6	1.3	1	0.8	0.4	No change
DIFFERENCE	1.6	1.3	1	0.8	0.4	No change
AMOUNT OF DO mg/L	8	6.5	5	4	2	0

TABLE NO.4.32

Culture no.KG3-NF-6T-48Z (with natural organic carbon source)

BURETTE READING	DAY 1(0HRS)	DAY 2 (24HRS)	DAY 3 (48 HRS)	DAY 4(72HRS)	DAY5(96 HRS)	DAY 6(120HRS)	DAY7 (144HRS)	DAY8 (168HRS)
INITIAL	0	0	0	0	0	0	0	0
FINAL	1.9	1.9	1.7	1.5	1.3	1.0	0.8	0.5
DIFFERENCE	1.9	1.9	1.7	1.5	1.3	1.0	0.8	0.5
AMOUNT OF DO mg/L	9.5	9.5	8.5	7.5	6.5	5	4	2.5

TABLE NO.4.33

Culture no.KG3-NF-6T-48Z (with artificial carbon source i.e. Glucose)

BURETTE READING	DAY 1(0HRS)	DAY 2 (24HRS)	DAY 3 (48 HRS)	DAY 4(72HRS)	DAY5(96 HRS)	DAY 6(120HRS)	DAY7(144HRS)	DAY8(168HRS)
INITIAL	0	0	0	0	0	0	0	0
FINAL	1.6	1.6	1.2	0.9	0.7	0.1	No change	No change
DIFFERENCE	1.6	1.6	1.2	0.9	0.7	0.1	No change	NO change
AMOUNT OF DO mg/L	8	8	6	4.5	3.5	0.5	0	0

GRAPHS:

FIG NO.4.3

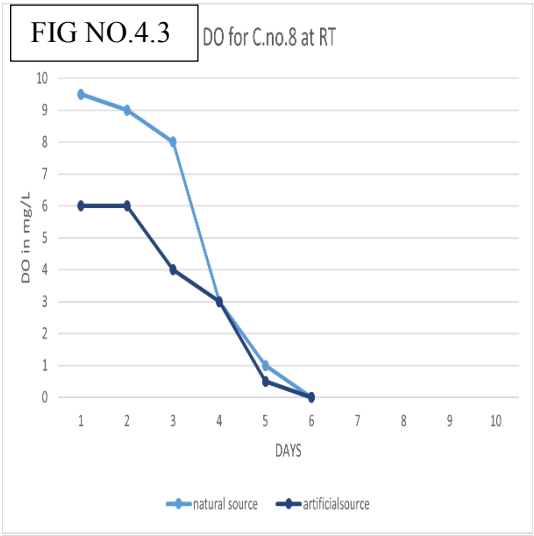


FIG NO.4.4

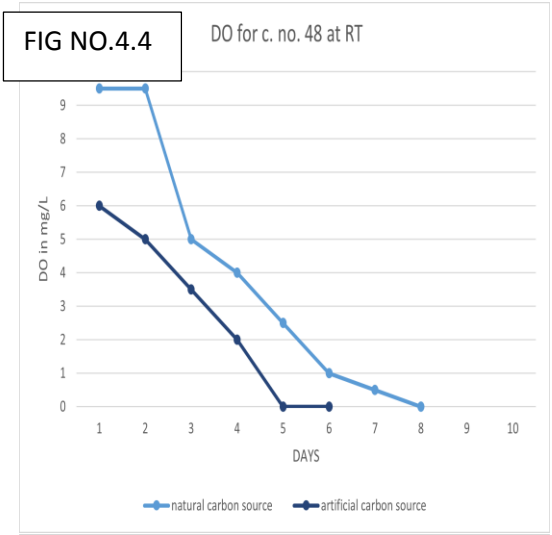


FIG NO.4.5

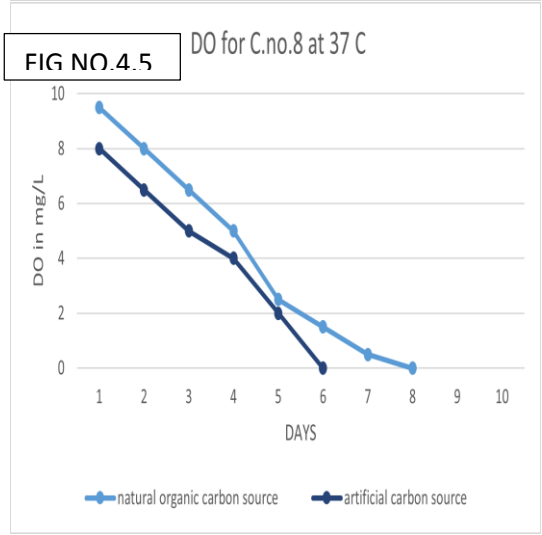
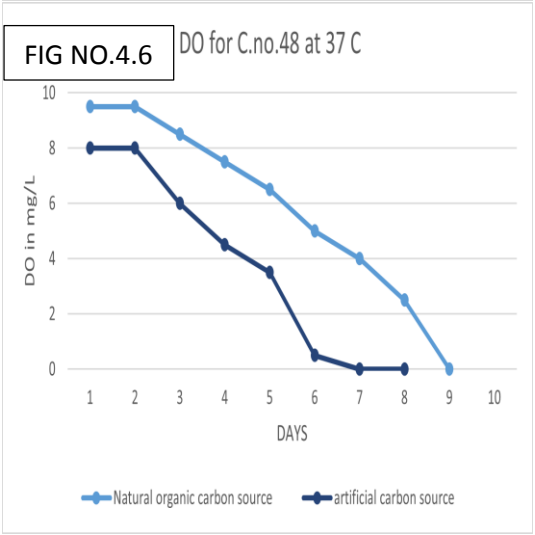


FIG NO.4.6



In the above graphs the bacterial respiration rate of the zooplankton associated bacteria was analysed using Winkler titration procedure for dissolved oxygen. The x-axis shows the no. of days the bacterial cultures were analysed. The Y-axis show the calculated DO in mg/L. It can be analysed that the dissolved oxygen decreases from the day-1 to day-8, concluding that the zooplankton associated bacterial cultures are utilising the dissolved oxygen for metabolic processes.

AMINO ACID MEASUREMENT USING NINHYDRIN:

Standard: Bovin serum albumin

Solvent: equal parts of water and n-propanol

Reagent: ninhydrin solution prepared in ethanol

For natural organic carbon source

TABLE NO. 4.34

Tube no.	Standard conc.(mg/ml)	Diluent (ml)	Final volume (ml)	Amount of Ninhydrin (ml)	Amount of solvent		OD At 570 nm
1	0.2	0.8	1	1	5		0.25
2	0.4	0.6	1	1	5		0.41
3	0.6	0.4	1	1	5	Incubation	0.67
4	0.8	0.2	1	1	5	At 90^0C	0.9
5	1	0	1	1	5	For 20	1.2
6	Organic carbon source	0	1	1	5	minutes	0.312
7	Artificial carbon source	0	1	1	5		0.1
8	KG3-NF-13T-8Z at RT(0hrs)	0	1	1	5		0.301
9	KG3-NF-13T-8Z at Rt(24hrs)	0	1	1	5		0.250

10	KG3-NF-13T-8Z at Rt(48hrs)	0	1	1	5		0.201
11	KG3-NF-13T-8Z at Rt(72hrs)	0	1	1	5	Incubation at 90^0C for 20 minutes	0.1
12	KG3-NF-13T-8Z at Rt(96hrs)	0	1	1	5		0.07
13	KG3-NF-13T-8Z at Rt(120hrs)	0	1	1	5		0.01
14	KG3-NF-13T-8Z at RT(0hrs)	0	1	1	5		0.2
15	KG3-NF-13T-8Z at RT (24hrs)	0	1	1	5		0.12
16	KG3-NF-13T-8Z at RT (48hrs)	0	1	1	5		0.09

17	KG3-NF-13T-8Z at RT (72hrs)	0	1	1	5		0.07
18	KG3-NF-13T-8Z at RT (96hrs)	0	1	1	5		0.04
19	KG3-NF-13T-8Z at RT (120hrs)	0	1	1	5		0.001
20	KG3-NF-RT-48Z at RT (0hrs)	0	1	1	5	Incubation at 90^0C for 20 minutes	0.301
21	KG3-NF-RT-48Z at RT (24hrs)	0	1	1	5		0.20
22	KG3-NF-RT-48Z at RT (48hrs)	0	1	1	5		0.17

23	KG3-NF-RT-48Z at RT (72hrs)	0	1	1	5		0.06
24	KG3-NF-RT-48Z at RT (96hrs)	0	1	1	5		0.003
25	KG3-NF-RT-48Z at RT (120hrs)	0	1	1	5	Incubation at 90^0C for 20 minutes	0.00
26	KG3-NF-RT-48Z at RT (0hrs)	0	1	1	5		0.110
27	KG3-NF-RT-48Z at RT (24hrs)	0	1	1	5		0.13
28	KG3-NF-RT-48Z at RT (48hrs)	0	1	1	5		0.07

29	KG3-NF-RT-48Z at RT (72hrs)	0	1	1	5		0.006
30	.KG3-NF-RT-48Z at RT (96hrs)	0	1	1	5	Incubation at 90^0C for 20 minutes	0.001
31	KG3-NF-RT-48Z at RT (120hrs)	0	1	1	5		0

GRAPH:

FIG. NO. 4.7: GRAPH OF STANDARD

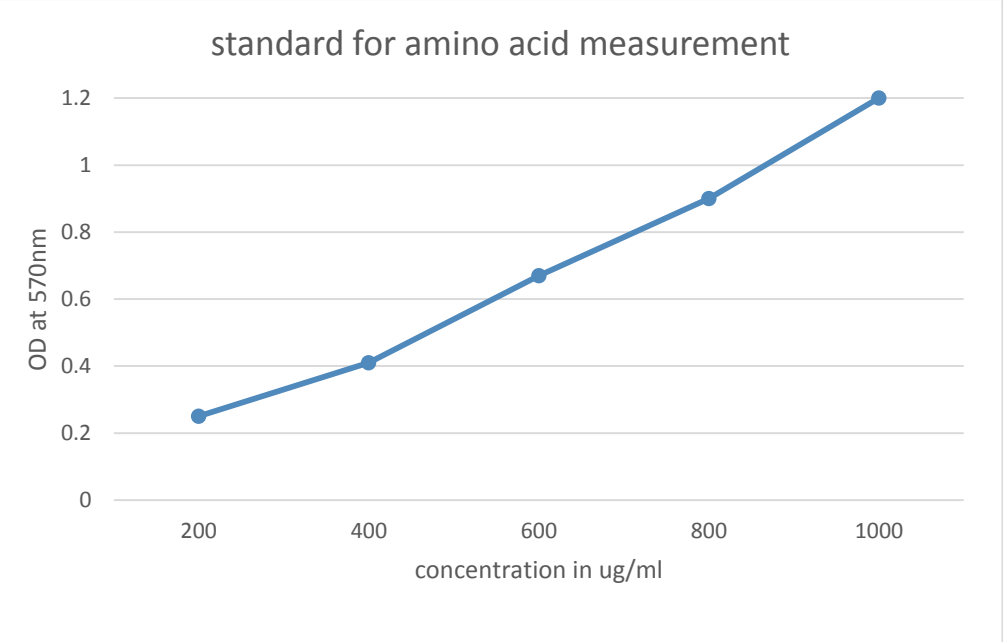
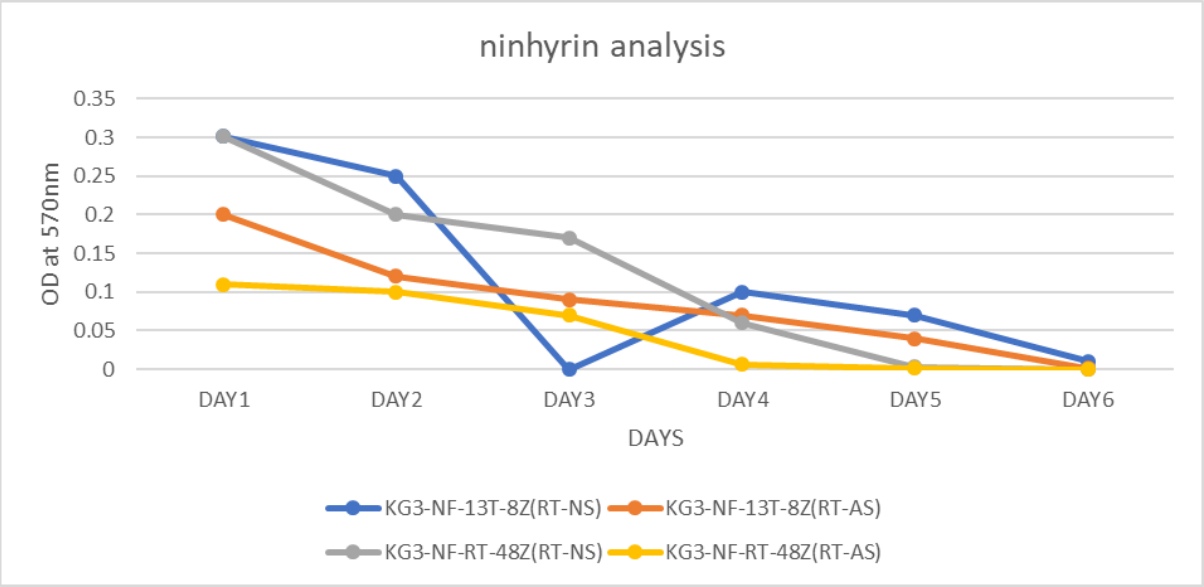


FIG. NO. 4.8: GRAPH FOR NINHYDRIN ANALYSIS



In the above graph the days are shown on the x-axis and OD at 570nm is shown on the y-axis. It can be concluded from above, that the free or bound amino group measurements are decreasing from day-1 to day-6 which can be confirmed by the decrease in Optical density. This could be due to the utilisation of available molecules for metabolic processes by the zooplankton associated bacteria used in the experiment.

Estuaries organic matter is mediated by the yearly monsoon runoffs from land and the effluents that are released by the industries or domestic waste or garbage leakages from the near shore dumps. In the study conducted on Indian tropical estuaries i.e. Godavari, India shows that bacterial metabolic rates and organic matter is usually affected by the monsoon.

The utilisation of organic carbon source is due to enzymes that are produced by the zooplankton associated bacteria that help them break down the complex organic carbon molecules to simple sugars that can be utilised very easily (Bickel et. al, 2014). We have screened the cultures for their amylase activity, where we can conclude that many of the bacterial isolates have good carbon utilisation capability. Along with that they show some positive results for chitinase, catalase, pectinase activity.

Having adopted experimental approach to determine the bacterial respiration rate our study shows that there was decreased in the dissolved oxygen level that was seen during the analysis of the same using Winkler titration. Decrease in salinity between initial (30%) and final (15%) was seen, which is determined by using Refractometer. This may be due to salt accumulation by the bacterial isolate to maintain their osmotic balance. Our observation implies that organic matter in the setup was utilised by the respective zooplankton associated bacteria. It can be compared with the earlier studies conducted by Siuda and Chrost (2000) who reported increase in bacterial respiration rate with increased particulate organic matter.

5.SUMMARY

The current study is carried out with an aim to isolate, enumerate and assess the organic carbon degradation potential of zooplankton associated bacteria, by analysis of the metabolic rates of selected isolated culture and also screening the cultures for their enzymatic activity. Total 50 zooplankton associated bacteria were isolated following Wang et.al.,2021 with some modifications. Their identification was done using biochemical tests such as sugar fermentation, indole test, methyl red test, TSI, catalase, oxidase, citrate test. Later all the isolates were screened for enzyme activity and the cultures showing good zone of clearance was selected. Two cultures i.e. KG3-NF-13T-8Z and KG3-NF-RT-48Z were short listed to check their metabolic rates by analyzing the bacterial respiration rate. Experimental setup was maintained as described in the protocols and respiration rate were measured after every 24hrs using Winkler titration, and experiment was continued till DO (dissolved oxygen was null). Along with the Dissolved oxygen periodic samples were taken from the experimental setup to check the amino acid measurement by ninhydrin test. The results show decrease in concentration of free amino acid with decreasing optical density. Decrease in salinity was seen when the initial sample of 0hrs and final day samples were checked using Refractometer. CHECK SENTENCE AND GRAMMER The reason could be accumulation of the salts from the environment to maintain their cell membrane integrity.

Estuaries receives organic matter (OM) from multiple sources, including terrestrial C3 and C4 plants, soil, freshwater, estuarine and marine phytoplankton, and domestic industrial sewage. Contribution of OM from the diverse source makes the OM cycling in estuaries more complex. Biogeochemical cycling of OM in aquatic systems is of global concern as it strongly influences the global carbon cycle by acting as a sink or source for the atmospheric carbon dioxide (CO2). Being highly productive zones estuaries can act as sink, or due to extensive processing of OM, they can be source of atmospheric carbon dioxide.

Zooplankton associated bacteria can be a saver in this case as it is already present in the gut of zooplankton. It has seen the extremities of the ocean and has developed itself to combat stressful conditions in the oceans. And hence, this potential of theirs can be utilised to tackle the situation in a smallest way possible and in a way that is environment friendly manner.

6. CONCLUSION

50 zooplankton associated bacterial cultures were isolated from zooplankton samples. Later biochemical tests were done to identify the cultures. The main focus was to select cultures that have good sugar utilization ability. These cultures were also screened for amylase activity. And out of 50 isolates, two cultures with good substrate i.e. sugar, utilisation were short listed for the bacterial respiration rate. And their potential of organic carbon degradation was checked by caring out the lab experiment. Culture no.KG3-NF-13T-8Z have shown good organic carbon degradation as compared to culture no. KG3-NF-RT-48Z. apart from that culture no.KG3-NF-13T-8Z has shown urease, catalase, protease, chitinase and amylase activity (zone of clearance 3cm). It is the only bacterial culture that has shown methyl red positive test.

Similarly, KG3-NF-RT-48Z has shown amylase activity positive, with a zone of clearance of 3.5cm after the media plates used for their activity were flooded with iodine solution.

Amino acid measurements were also carried during the course of the experiment, using ninhydrin test and taking the OD at 570nm. There were declining variations seen.

From the entire results we can conclude that there was decrease in Dissolved Oxygen level seen in the incubated DO bottles with the zooplankton-associated bacterial cultures confirmed by the Winkler titration for the determination of bacterial respiration rate at continuous interval of 0hrs, 24hrs,48hrs, etc. hence, we can say that the zooplankton associated bacteria has some potential in degrading the organic carbon. The dissolved oxygen decreases in natural organic carbon source comparatively faster than artificial carbon source. The dissolved oxygen was zero on the day 6 of experiment where natural organic carbon source was used for inoculation.

This may be due to the high respiratory rate in natural organic carbon source which has nutrients and salts usually essential for the zooplankton associated bacteria.

The KG3-NF-13T-8Z cultural isolate showed the highest respiration rate with natural organic carbon source incubated at room temperature as compared to the KG3-NF-RT-48Z cultural isolate.

7.FUTURE PROSPECTS

- the isolated cultures can be used to extraction of pigments and lipids.

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APPENDIX

APPENDIX-1

(MEDIA)

TABLE NO.35

1)ZOBELL MARINE AGAR

COMPOSITION	GRAM'S / LITRE
Peptone	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

Ph (at 25^0 C)	7.6 +/- 0.2

55.25g is suspended in 1000ml filtered sea water/distilled. Dissolve the media by keeping in water bath. Sterilize by autoclaving at 15lbs pressure (121^0 C) for 15 minutes. Mix well and pour in sterile petri plates

MEDIA FOR BIOCHEMICAL TESTS

	Gram's/litre
TRYPTONE WATER	
Tryptone	10g/L
Sodium chloride	5g/L
Distilled water	1L

GLUCOSE PEPTONE BROTH	
Buffered peptone	7g/L
Dextrose	5g/L
Dipotassium phosphate	5g/L
Ph	6.9-0.2

SIMMON'S CITRATE AGAR

Magnesium sulphate	0.2 g/litre
Mono ammonium phosphate	1.0 g/litre
Dipotassium phosphate	1.0 g/litre
Sodium Citrate	2.0 g/litre
Agar	5.0 g/litre
Bromothymol blue	15.0 g/litre
Ph (at 25°c)	6.8±0.2

SEMISOLID NUTRIENT AGAR

Peptone	5g/L
Yeast extract	1.5g/L
Beef extract	1.5g/L
Sodium chloride	5g/L
Agar	7g/L
Ph	7.4±0.2

CHRISTENSEN'S UREA AGAR

Peptone	1.0 g
Dextrose	1.0 g
Sodium Chloride	5.0 g
Dipotassium Phosphate	1.2 g

Mono-potassium phosphate	0.8 g
Phenol Red	0.012 g
Agar	15.0 g
Final Ph (at 25^0 C)	7.4±0.2

TRIPLE SUGAR IRON (TSI)

Peptone	10 g/litre
Tryptone	10 g/litre
Yeast extract	3 g/litre
Beef extra	3g/litre
Lactose	10 g/litre

APPENDIX-II

REAGENTS: 1) 1% -tetra-methyl-p-phenylenediamine dihydrochloride

COMPOSITION	GRAMS
Tetra-methyl-p-phenylenediamine dihydrochloride	1
Distilled water	100ml

2)1%- Congo red solution

COMPOSITION	GRAMS
Congo red	1
Distilled water	100ml

3)1 M NaCl

COMPOSITION	GRAMS
NaCl	58.44
Distilled water	1L

4)Iodine solution

COMPOSITION	GRAMS
I_2	0.3
KI	0.6
Distilled water	100ml