

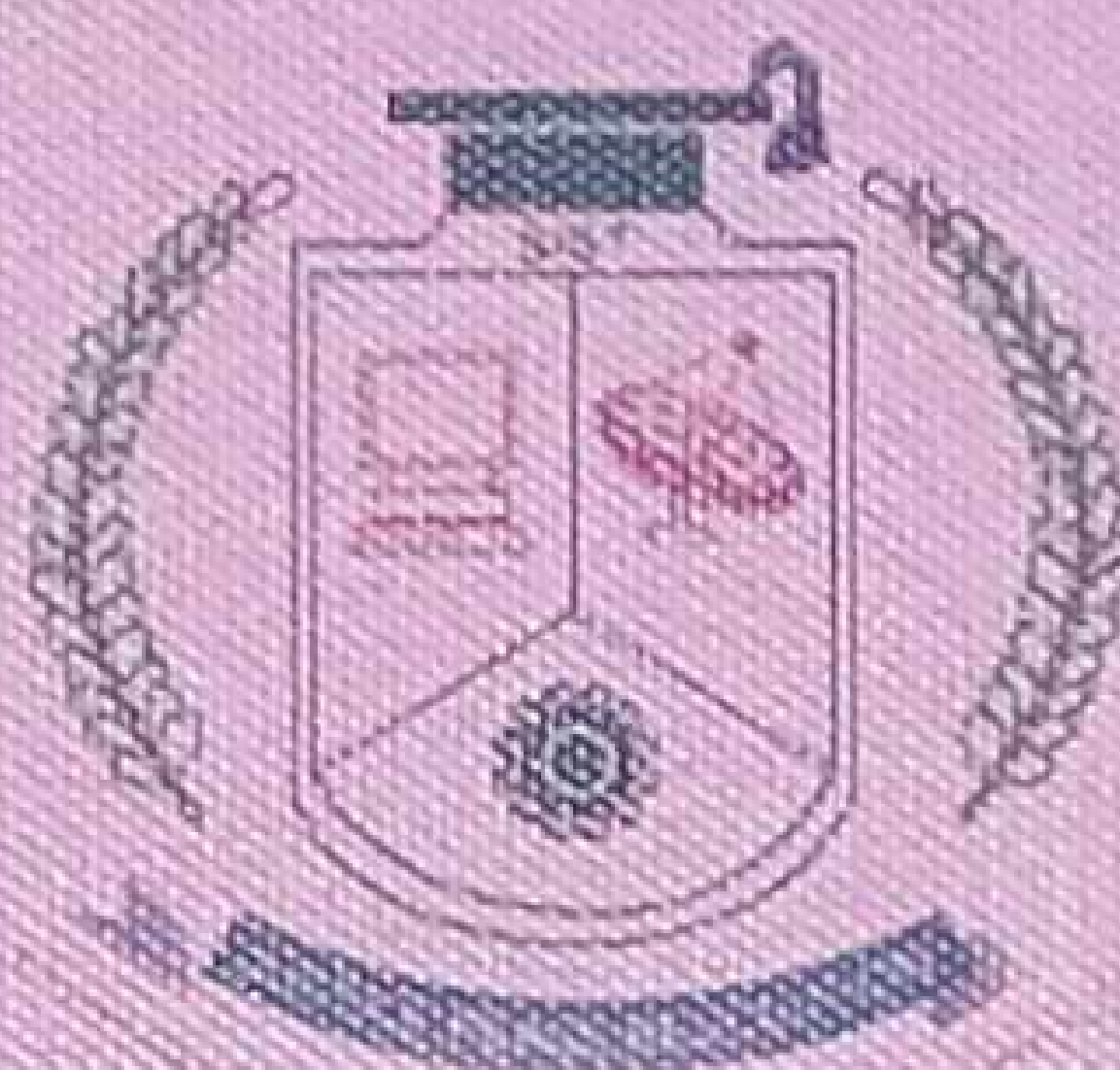
BIOPROSPECTING OF POTENTIAL BACTERIA FROM VARIOUS SOIL SAMPLES

PROJECT REPORT

Submitted in partial fulfilment of the requirements for the award of a Bachelor of
Science degree in Microbiology

By

ABIEZER JIOE RAJA R (Reg No. 41300001)



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

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
CATEGORY -1 UNIVERSITY BY UGC**

**Accredited with Grade "A++" by NAAC | 12B Status by UGC | Approved
by AICTE**

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

APRIL - 2024

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of ABIEZER JIOE RAJA R (Reg No. 41300001) who carried out the project entitled "BIOPROSPECTING OF BACTERIA FROM VARIOUS SOIL SAMPLES" under my supervision from September 2023 to April 2024.

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Dr. V. RAMESH KUMAR

Submitted for Viva voce Examination held on 26.04.2024

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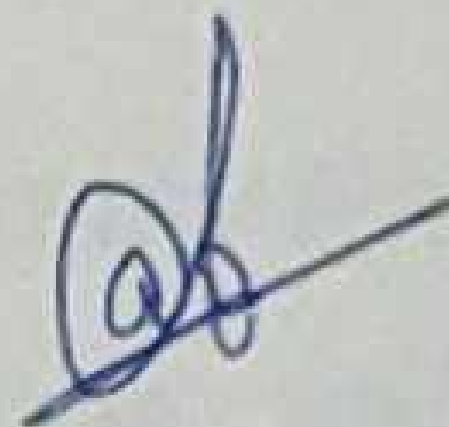
External Examiner

DECLARATION

I ABIEZER JIJOE RAJA R, R (Reg No. 41300001) hereby declare that the Project Report entitled "BIOPROSPECTING OF POTENTIAL BACTERIA FROM VARIOUS SOIL SAMPLE" done by me under the guidance of Dr. D. SARAVANAN, Assistant professor (Research), Centre for laboratory animal technology and research, Sathyabama Institute of Science and Technology is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Microbiology.

DATE: 24-04-2024

PLACE: CHENNAI


SIGNATURE OF THE CANDIDATE

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I am pleased to acknowledge my sincere thanks to Board of Management of **SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

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ABSTRACT

Bioprospecting, the exploration and sustainable utilization of biological resources, has emerged as a critical avenue for discovering valuable genetic and biochemical materials. This process involves screening diverse biological samples, including microorganisms from various environments, to uncover novel compounds with commercial potential. Enzymes, abundant in marine ecosystems and soil, play a pivotal role in bioprospecting endeavours due to their catalytic properties. Azo dyes, frequently utilized in research, serve as model substrates for studying microbial degradation mechanisms, offering insights into environmental bioremediation strategies. Additionally, the production of antimicrobial compounds by bacteria underscores the significance of microbial diversity in combating antimicrobial resistance. Terrestrial and aquatic ecosystems, such as mangroves and marine environments, harbour vast microbial communities crucial for ecosystem health. However, anthropogenic activities pose significant threats to these ecosystems, highlighting the need for comprehensive studies on microbial communities and their ecological roles. This abstract encapsulates the multifaceted significance of microbial diversity and bioprospecting in ecological conservation and industrial innovation.

KEYWORDS: Bioprospecting, Enzyme activity, Dye degradation activity, antimicrobial activity.

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LIST OF ABBREVIATIONS AND SYMBOLS

gm	-	Grams
ml	-	Millilitre
µl	-	Microlitre
m	-	Meter
%	-	Percentage
km	-	Kilometer
ha	-	Hectare
°C	-	Degree Celsius
Hrs	-	Hours
Atm	-	Atmospheres

categorized under the genera *Bacillus* and *Paenibacillus*. In summary, the study provides an understanding of the isolation and characterization of bacterial strains that produce the amylase enzyme. These findings have practical applications in industrial settings where amylase enzyme is required for the production of various products.

Saranraj, *et al.*, (2011) characterized that under aerobic circumstances, the enzyme azoreductase totally breaks down azo dyes. There is no prior treatment performed before collecting and storing the dye effluent. The pour plate technique is used to extract the bacterium that decolorizes dyes. Colonies are then selected and streaked for purity. The strains underwent the biochemical test while being stored while being preserved in the nutrient agar medium. putting an inoculum of bacteria in a flask and shaking it with a machine for four days. The dye solution is collected every 24 hours, and a decolorization and biodegradation assay is conducted. The deterioration of the dye is evident in a shift in colour from black to brown.

Saravanan, *et al.*, (2015) studied Dye-degrading bacteria isolated from textile effluent hold potential applications in bioremediation and wastewater treatment processes. These bacteria can be utilized in bioreactors, constructed wetlands, and bioaugmentation strategies to enhance the degradation of dye pollutants in wastewater treatment plants. However, challenges such as substrate specificity, process optimization, and microbial competition need to be addressed to maximize the efficacy of bacterial-based treatment systems.

Sahoo and dhal, *et al.*, (2009) showed microorganisms in aquatic environments play crucial roles in nutrient cycling, carbon sequestration, and the degradation of organic matter. Additionally, they contribute to the production of oxygen through photosynthesis and serve as primary producers in aquatic food webs. However, human activities, particularly in coastal areas, pose significant threats to microbial processes and water quality. Pollution from industrial effluents, agricultural runoff, and urbanization

can disrupt microbial communities, leading to imbalances in ecosystem dynamics and degradation of water quality. Efforts to understand and conserve microbial diversity in aquatic environments are essential for maintaining ecosystem health and ensuring sustainable water resources. Collaborative research initiatives focusing on microbial ecology, biogeochemistry, and ecosystem management are crucial for addressing emerging challenges and mitigating the impacts of human-induced disturbances on aquatic microbial communities.

Sahoo and dhal, *et al.*, (2008) characterized by their unique intertidal habitats, harbour a rich diversity of microorganisms that play essential roles in nutrient recycling and ecosystem functioning. This review explores the potential microbial diversity within mangrove ecosystems, focusing on bacteria, fungi, and actinomycetes, and their contributions to nutrient cycling and environmental processes. Mangrove forests, spanning approximately 181,000 km² across 112 countries and territories, represent vital coastal ecosystems that support diverse microbial communities. These microbes thrive in the dynamic interplay of saltwater and freshwater environments, where they play key roles in decomposing organic matter, cycling nutrients, and maintaining ecosystem health. Microbes inhabiting mangrove ecosystems are adept at breaking down complex organic compounds present in dead mangrove vegetation, thereby releasing essential nutrients such as nitrogen, phosphorus, and others. This nutrient recycling process is crucial for sustaining mangrove vegetation and supporting the diverse array of flora and fauna that depend on these ecosystems for survival.

Research conducted by Mindy G brown, *et al.*, (2009) investigated antibiotic resistance profiles of bacteria isolated from deep terrestrial subsurface environments. Through molecular and phenotypic analyses, the study revealed widespread resistance to multiple classes of antibiotics among subsurface bacterial isolates, including resistance to commonly used antibiotics such as penicillin, tetracycline, and erythromycin. Furthermore, the presence of mobile genetic elements carrying antibiotic resistance genes suggested the potential for horizontal gene transfer, facilitating the spread of

resistance determinants within microbial communities. These findings underscore the importance of understanding the mechanisms driving antibiotic resistance in deep subsurface bacteria. Factors such as anthropogenic contamination, natural selection pressure, and genetic exchange mechanisms may contribute to the dissemination of resistance genes in these environments. Additionally, the presence of diverse microbial communities in deep subsurface environments highlights the potential reservoir of antibiotic resistance genes that could impact human health through environmental contamination or transmission to pathogens.

Saravanan, *et al.*, (2012) studied the forest ecosystem represents a vast and diverse reservoir of microbial life, including bacteria with potential antimicrobial properties. Harnessing the microbial diversity from these ecosystems could lead to the discovery of novel antimicrobial compounds. This research aims to explore the existing research on the exploitation of bacteria derived from forest environments for the discovery of antimicrobial agents. Various studies investigating the isolation, characterization, and evaluation of antimicrobial activity of bacteria from forest ecosystems are reviewed, highlighting their potential in drug discovery and development. Additionally, challenges and future prospects in this field are discussed.

Jianbo Guo, *et al.*, (2010) characterized that the redox mediators, such as electron shuttles (ES), enable microbial energy metabolism by transferring electrons from electron donor substances to microbes or from microbes to electron acceptor substances. It is possible to artificially induce ES, which results in electron flow and microbial energy metabolism. As a result, ES can be induced to breakdown azo dye. There are various reduction processes, including chemical, indirect/mediated, and direct enzymatic reduction. By including redox-mediating substances like anthraquinonesulfonate (AQS) and anthraquinonedisulfonate (AQDS), the azo degradation can be started. Aerobic bacteria with azoreductase, which may breakdown azo dyes, have been found, including K22 and KF46. After purification, these bacteria no longer contain flavin.

CHAPTER - 3

AIM AND OBJECTIVE

3.1 AIM

To study the bioprospecting of potential bacteria from various soil samples.

3.2 OBJECTIVE

- Collection of soil samples from different environment.
- Isolation of morphologically different bacteria.
- Screening for extracellular enzyme.
- Determination of biodegradation of dye by bacterial isolates.
- Screening for antimicrobial activity of bacterial isolates.

CHAPTER – 4

MATERIALS AND METHODS

4.1 COLLECTION OF SOIL SAMPLES

Soil samples were collected from various area such as Thoothukudi sea (lat. 8.7644° N, long. 78.1507° E), Thoothukudi salt pan (lat. 8.7745° N, long. 78.1279° E), Pichavaram mangrove (lat. 11.4226° N, long. 79.7748° E), Thamirabarani river (lat. 8.601962° N, long. 77.264131° E), Sembur terrestrial (lat. 12.4813° N, long. 80.0598° E). In all locations, few grams of soil samples were transferred to sterile polythene bags with sterile spatula and transport to laboratory for process. Saravanan, *et al.*, (2012)

4.2. ISOLATION AND CHARACTERIZATION OF BACTERIA

The collected soil samples were subjected to serial dilution for the isolation and enumeration of culturable bacterial populations. This process involved diluting one gram of soil sample in 9 ml of sterile distilled water, resulting in dilutions ranging from 10^{-1} to 10^{-7} . After dilution, 0.1 ml of each diluted sample was aseptically transferred onto nutrient agar plates. The spread plate method was employed to ensure uniform distribution of the bacterial suspension on the agar surface, using a sterile L-shaped rod. The plates were then incubated at room temperature for a period of 48 to 72 hours to allow for bacterial growth. Following incubation, morphologically distinct bacterial colonies were selected from the nutrient agar plates. These colonies were characterized based on their visual appearance, such as size, shape, colour, and texture. Selected colonies were then purified by streaking them onto fresh nutrient agar plates to obtain pure cultures, ensuring the isolation of individual bacterial strains. Subsequently, all purified isolates were sub-cultured and maintained on nutrient agar slants. These slants were stored at a temperature of 4°C to preserve the viability of the bacterial cultures for further analysis and experimentation.

4.3. DETERMINATION OF ENZYMATIC ACTIVITIES OF SOIL BACTERIA

All bacterial isolates obtained from the soil samples were subjected to screening for enzymatic activities, specifically for amylase and protease, using the agar plate method. This screening process aimed to identify bacterial strains capable of producing these enzymes, which have significant industrial and biotechnological applications. The procedure involved spot inoculating each bacterial isolate onto specific enzyme screening media designed to detect amylase and protease activities. These media were supplemented with substrates that could be hydrolyzed by the respective enzymes, such as starch for amylase and casein for protease. After inoculation, the plates were incubated at a temperature of 28°C for a duration of 48 hours to allow for bacterial growth and enzyme production. A control plate, which remained uninoculated, was also included to serve as a reference for comparison. Enzymatic activity was determined by visual inspection of the plates after the incubation period. The presence of a zone of clearance or a zone of colour change around the bacterial colonies indicated the hydrolysis of the substrate by the enzymes produced by the bacterial isolates. In some cases, developing reagents may have been added to the plates to enhance the visualization of enzymatic activity. This screening approach enabled the researchers to identify bacterial isolates with the potential to produce amylase and protease enzymes. These enzymes are of significant interest due to their role in various industrial processes, including food processing, detergent manufacturing, and biofuel production. By screening a diverse range of bacterial isolates from the forest ecosystem, Gayathri, *et al.*, (2010). aimed to uncover novel enzyme-producing strains that could be further characterized and utilized for biotechnological applications.

Table 4.1 Screening mediums and interpretation of extracellular enzymes

Enzyme	Media	Method of Detection	Reagents used
Amylase	Starch Agar	Zone of clearance	1% Iodine
Protease	Skim Milk Agar	Zone of clearance	-

4.4. DETERMINATION OF BIO DEGRADATION ACTIVITIES OF SOIL BACTERIA

Screening process was employed to assess the dye-degrading activity of bacterial isolates obtained from the soil samples. The study aimed to identify bacterial strains capable of degrading various dyes, including malachite green, safranin, and crystal violet, using the agar plate method. Each bacterial isolate was spot inoculated onto specific screening media plates supplemented with the respective dyes as substrates. These screening media plates were designed to provide a favourable environment for the growth of bacteria and the degradation of the dye molecules. Following inoculation, the plates were then incubated at room temperature for a period of 48 to 72 hours to allow for bacterial growth and potential dye degradation. An uninoculated plate served as a control to account for any non-specific changes in the medium. After the incubation period, morphologically distinct colonies were observed on the screening media plates. Colonies exhibiting a zone of clearance or discoloration around them were selected as potential dye-degrading strains. The presence of a clear zone indicated that the bacteria had the enzymatic capability to degrade the dye molecules present in the medium. The determination of dye-degrading activity was primarily based on visual inspection, where the presence of a distinct zone of clearance around bacterial colonies was indicative of dye degradation. This screening approach allowed for the rapid identification of bacterial isolates with the potential to degrade malachite green, safranin, and crystal violet dyes. The identification of bacterial strains capable of dye degradation is of significant importance due to the environmental implications associated with dye pollution. Dye-degrading bacteria hold promise for the bioremediation of dye-contaminated environments and the development of eco-friendly solutions for wastewater treatment. By screening a diverse range of bacterial isolates from the forest ecosystem, aimed to identify novel strains with potential applications in dye degradation and environmental remediation.

Table 4.2 Screening mediums and interpretation of dye degradation

Dye	Media	Method of detection
Malachite green	Nutrient agar + 10mg per 100ml of malachite green dye.	Zone of clearance
Safranin	Nutrient agar + 10mg per 100ml safranin.	Zone of clearance
Crystal violet	Nutrient agar 10mg per 100ml crystal violet.	Zone of clearance

4.5. DETERMINATION OF ANTI MICROBIAL ACTIVITIES OF SOIL BACTERIA

The antimicrobial activity of bacterial isolates obtained from the ecosystem was assessed using the agar well diffusion method. Prior to testing, all bacterial isolates were cultured in nutrient broth and incubated for 48 hours to ensure robust growth. Subsequently, a 10% inoculum of each bacterial culture was transferred into fermentation medium and incubated in a rotary shaker at 28°C for 120 hours to promote the production of antimicrobial compounds. After the fermentation period, the culture broth was centrifuged at 10,000 rpm for 10 minutes to separate the bacterial cells from the supernatant containing the antimicrobial compounds. The cell-free supernatant was then collected and stored in sterile vials for further analysis.

To evaluate the antimicrobial activity of the cell-free supernatant, a panel of test bacterial strains was used, including *Vibrio cholerae*, *Aeromonas*, *Staphylococcus aureus*, *Klebsiella*, and *Candida*. These strains represent clinically relevant pathogens commonly associated with infectious diseases. To perform the agar well diffusion assay, bacterial growth from each test strain was standardized to a 0.5 McFarland standard and inoculated onto Mueller Hinton agar plates using sterile cotton swabs. Wells with a diameter of approximately 5 mm were then made in the agar plates using a sterile cork borer. Subsequently, 100µl of the cell-free

supernatant from each bacterial isolate was added into the wells. The plates were then incubated at 37°C for 24 hours to allow for the diffusion of antimicrobial compounds from the supernatant into the agar medium. After incubation, the plates were observed for the presence of zones of inhibition around the wells, indicating the antimicrobial activity of the cell-free supernatant against the test bacterial strains. The diameter of the inhibition zones was measured and recorded as an indicator of the potency of the antimicrobial compounds produced by the bacterial isolates. This methodology allowed for the rapid screening and assessment of the antimicrobial potential of bacterial isolates derived from the ecosystem. By employing a diverse panel of test bacterial strains, Valgas, *et al.*, (2007) aimed to evaluate the broad-spectrum antimicrobial activity of the cell-free supernatant and identify potential candidates for further investigation and development.

CHAPTER – 5

RESULTS AND DISCUSSION

5.1 COLLECTION OF SOIL SAMPLES

Soil samples were collected from various area such as Thoothukudi sea (lat. 8.7644° N, long. 78.1507° E), Thoothukudi salt pan (lat. 8.7745° N, long. 78.1279° E), Pichavaram mangrove (lat. 11.4226° N, long. 79.7748° E), Thamirabarani river (lat. 8.601962° N, long. 77.264131° E), Sembur terrestrial (lat. 12.4813° N, long. 80.0598° E). In all locations, few grams of soil samples were transferred to sterile polythene bags with sterile spatula and transport to laboratory for process. Saravanan, *et al.*, (2012) (Table 5.1) (Fig 5.1).

Table 5.1 Collection of soil samples

S. No.	Sampling Area	Sample Code
1	Thoothukudi sea	THS
2	Thoothukudi salt pan	THSP
3	Pichavaram mangrove	M
4	Thamirabarani river	TR
5	Sembur terrestrial	SEM



Fig 5.1 Soil samples from different areas

5.2. ISOLATION AND CHARACTERIZATION OF BACTERIA FROM SOIL

Totally 75 morphologically different bacterial colonies were isolated from the soil sample in which 7 isolates were from Thoothukudi sea (THS), 24 isolates from Thoothukudi salt pan (THSP), 13 isolates from Pichavaram mangrove (M), 19 isolates from Thamirabarani river (TR), 12 isolates from Sembur terrestrial (SEM). The bacterial population in the collected soil samples was ranged between 1.3×10^3 and 1.9×10^5 cfu/gm of soil. Majority of the bacterial isolates produced non pigmented colonies on nutrient agar. Of the 75 isolates 26 were pigmented (34.6%) and 34 (65.3%) were non-pigmented isolates. Pigments include yellow, orange and white. (Fig 5.2) (Table 5.2). In Previous study Saravanan, *et al.*, (2015) isolated bacteria from soil samples collected from Kodaikannal forest.

Table 5.2 Isolation of bacteria from different soil samples

S.NO	Sample Name	No. of isolates
1	THS	7
2	THSP	24
3	M	13
4	TR	19
5	SEM	12
TOTAL		75

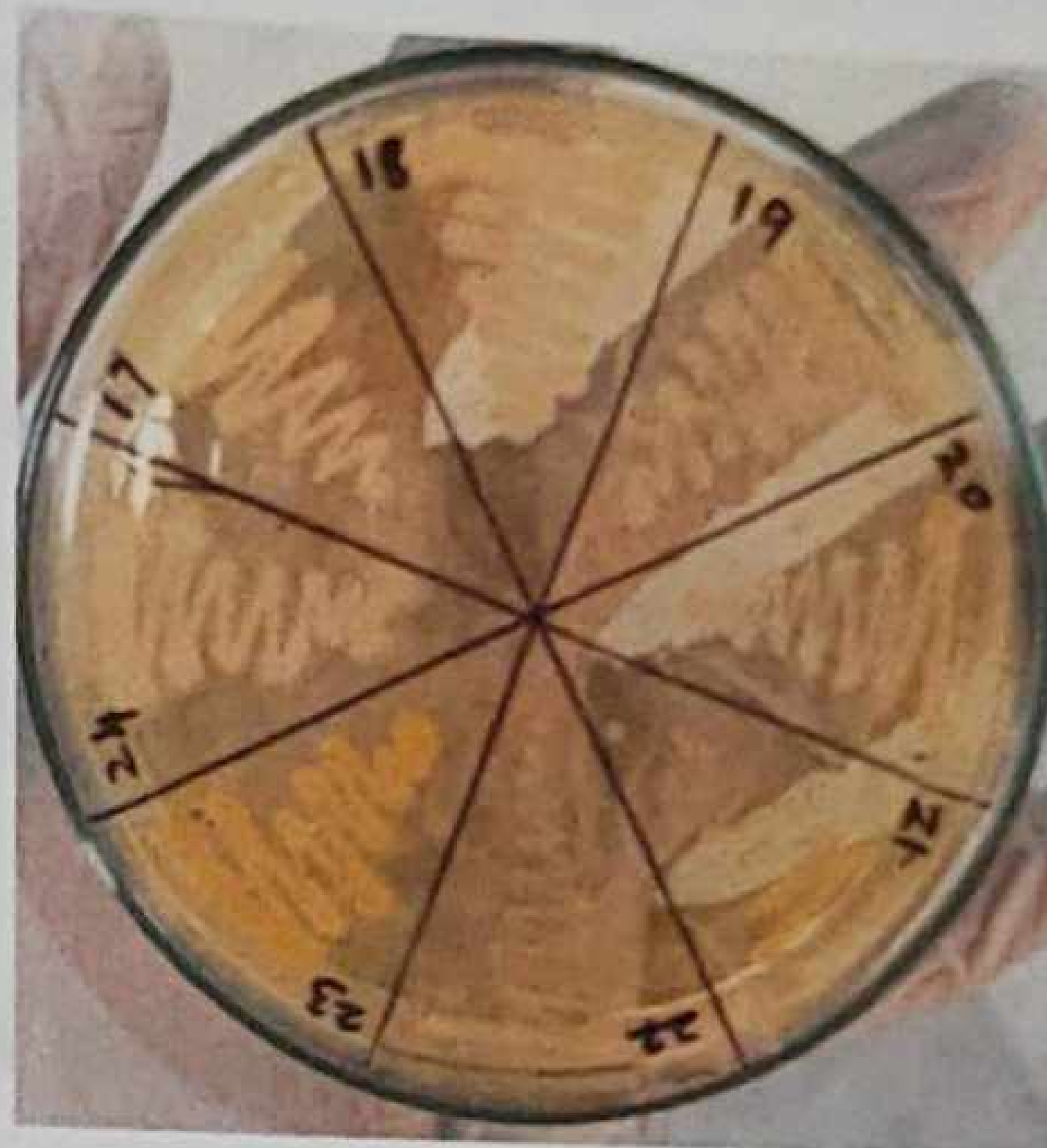


Fig 5.2 Isolation of bacterial isolates from soil samples

5.3. DETECTION OF ENZYMATIC ACTIVITIES OF SOIL BACTERIA

In the current study, an exhaustive enzyme screening was conducted on bacterial isolates derived from soil samples collected from diverse ecological niches, including Thoothukudi sea, Thoothukudi salt pan, Pichavaram mangrove, Thamirabarani river, and Sembur terrestrial environments. Enzymatic screenings are pivotal for understanding the metabolic potential and functional diversity of microbial communities within soil ecosystems.

Remarkably, all 75 bacterial isolates exhibited one or more enzymatic activities, indicating the broad enzymatic repertoire present within the sampled soil microbiota. This comprehensive enzymatic profiling provides valuable insights into the metabolic capabilities of the bacterial isolates and their potential roles in biogeochemical processes.

Among the enzymatic activities screened, amylase activity was prevalent, with 37 isolates demonstrating this enzymatic function. Amylases are crucial enzymes involved in the hydrolysis of starch and glycogen, breaking down complex polysaccharides into simpler sugars such as glucose and maltose. The prevalence of amylase activity suggests the importance of carbohydrate metabolism within the soil microbial community, potentially contributing to organic matter degradation and nutrient cycling processes.

Furthermore, protease activity was observed in 15 bacterial isolates, highlighting their ability to hydrolyze proteins into peptides and amino acids. Proteases play a vital role in nitrogen mineralization and organic matter decomposition, contributing to nutrient cycling and soil fertility. The presence of protease activity underscores the diverse metabolic strategies employed by soil bacteria to utilize complex organic substrates for growth and energy acquisition (Fig 5.3). (Table 5.3). In previous study by Praveen Kumar, *et al.*, (2015) screened various enzymatic activities in soil collected from Northern Tamil Nadu.

Table 5.3 Number of isolates showing positive for enzymes

S.NO	Enzymes	No. of Positive Isolates
1	Amylase	37
2	Protease	15



(a) Amylase



(b) Protease

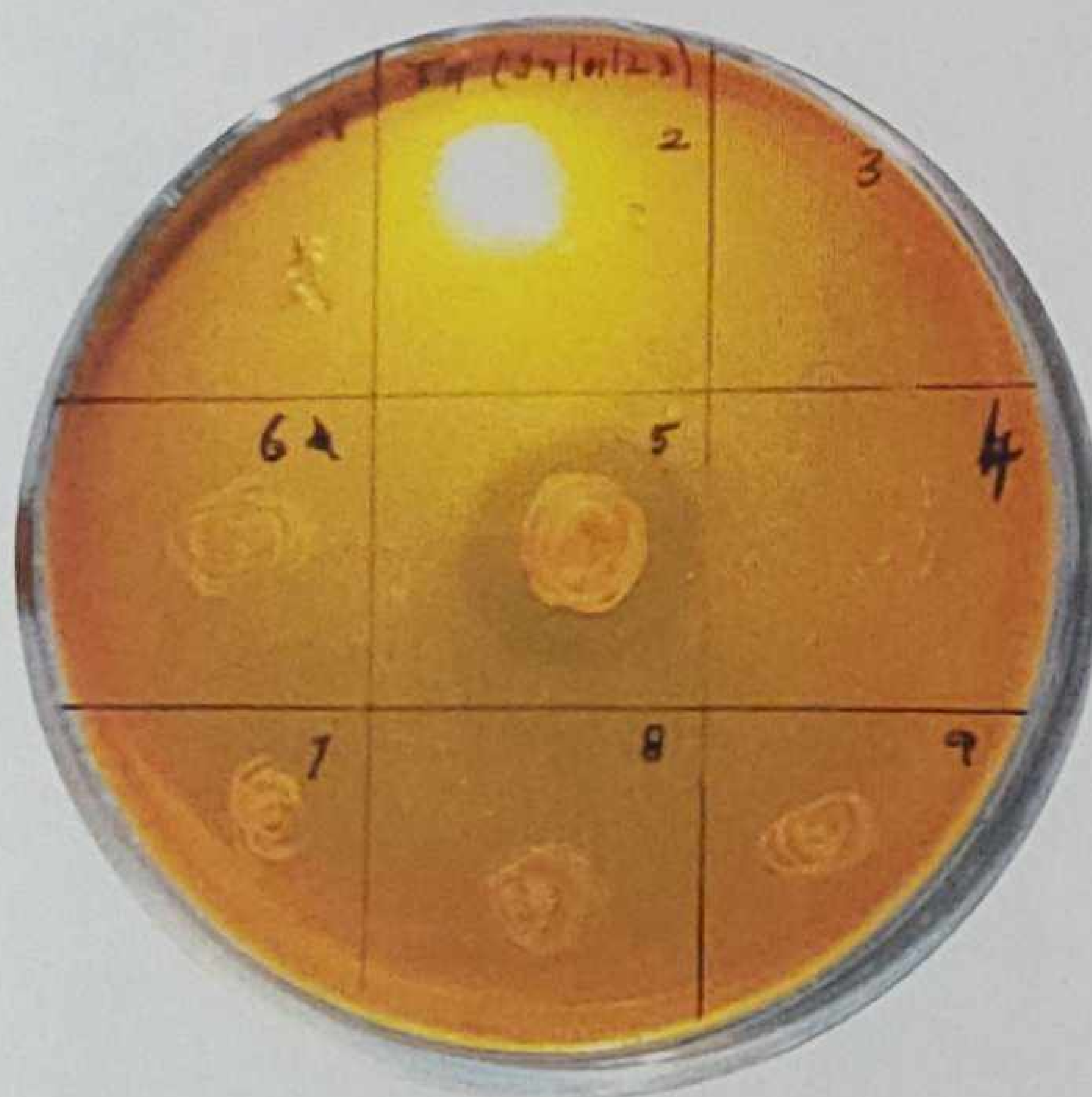


Fig 5.3 Enzyme activity by bacterial isolates

Table 5.4 Isolates report for enzymes

Si. NO	Strain No	Screening code	Amylase	Protease
1	THSP1	A1	-	-
2	THSP2	A2	-	-
3	THSP3	A3	-	-
4	THSP4	A4	-	-
5	THSP5	B1	-	-
6	THSP6	B2	-	+
7	THSP7	B3	+	-
8	THSP8	B4	-	-
9	THSP9	C1	+	-
10	THSP10	C2	+	-
11	THSP11	C3	+	-
12	THSP12	C4	+	-
13	THSP13	D1	-	-
14	THSP14	D2	+	-
15	THSP15	D3	+	-
16	THSP16	D4	-	-
17	THSP17	A1	-	-
18	THSP18	A2	+	-
19	THSP19	A3	+	-
20	THSP20	A4	+	-
21	THSP21	B1	+	-
22	THSP22	B2	+	-
23	THSP23	B3	+	-
24	THSP24	B4	+	-
25	SEM25	C1	-	-
26	SEM26	C2	-	-
27	SEM27	C3	+	-
28	SEM28	C4	-	-
29	SEM29	D1	-	-

30	SEM30	D2	-	-
31	SEM31	D3	-	-
32	SEM32	D4	-	-
33	SEM33	A1	-	-
34	SEM34	A2	-	-
35	SEM35	A3	-	+
36	SEM36	A4	+	-
37	M37	B1	+	-
38	M38	B2	+	-
39	M39	B3	+	-
40	M40	B4	-	+
41	M41	C1	+	-
42	M42	C2	+	+
43	M43	C3	-	-
44	M44	C4	+	-
45	M45	D1	+	-
46	M46	D2	-	-
47	M47	D3	-	-
48	M48	D4	+	-
49	M49	A1	+	-
50	TR50	A2	-	-
51	TR51	A3	+	+
52	TR52	A4	+	-
53	TR53	B1	-	+
54	TR54	B2	+	+
55	TR55	B3	+	+
56	TR56	B4	-	-
57	TR57	C1	-	-
58	TR58	C2	+	+
59	TR59	C3	-	+
60	TR60	C4	+	+
61	TR61	D1	-	-
62	TR62	D2	+	+

63	TR63	D3	+	+
64	TR64	D4	+	+
65	TR65	A1	+	+
66	TR66	A2	+	+
67	TR67	A3	-	+
68	TR68	A4	-	+
69	THS69	B1	+	-
70	THS70	B2	-	+
71	THS71	B3	-	-
72	THS72	B4	-	-
73	THS73	C1	-	-
74	THS74	C2	-	+
75	THS75	C3	+	+

5.4. DETECTION OF BIO DEGRADATION ACTIVITIES OF SOIL BACTERIA

In the current study, a comprehensive screening for dye degradation activities was conducted on bacterial isolates obtained from soil samples collected across various ecological niches, including Thoothukudi sea, Thoothukudi salt pan, Pichavaram mangrove, Thamirabarani river, and Sembur terrestrial environments. Dyes are synthetic compounds widely used in various industries, and their release into the environment poses environmental concerns due to their persistence and potential toxicity. Bacterial-mediated degradation of dyes represents a promising approach for remediation of dye-contaminated environments.

Remarkably, all 75 bacterial isolates exhibited one or more dye degradation activities, underscoring the widespread distribution of dye-degrading microorganisms within the sampled soil microbiota. This comprehensive screening provides valuable insights into the metabolic versatility of soil bacteria and their potential for environmental remediation.

Among the dye degradation activities screened, 26 isolates exhibited crystal violet degradation activity, indicating their ability to degrade this commonly used dye. Crystal violet is a cationic dye often employed in textile industries, and its degradation by bacteria represents an important mechanism for mitigating environmental contamination.

Furthermore, 15 isolates exhibited malachite green degradation activity, highlighting their capacity to degrade this dye, which is widely used in the textile and aquaculture industries. Malachite green degradation by bacteria is crucial for reducing its environmental impact and preventing its accumulation in aquatic ecosystems.

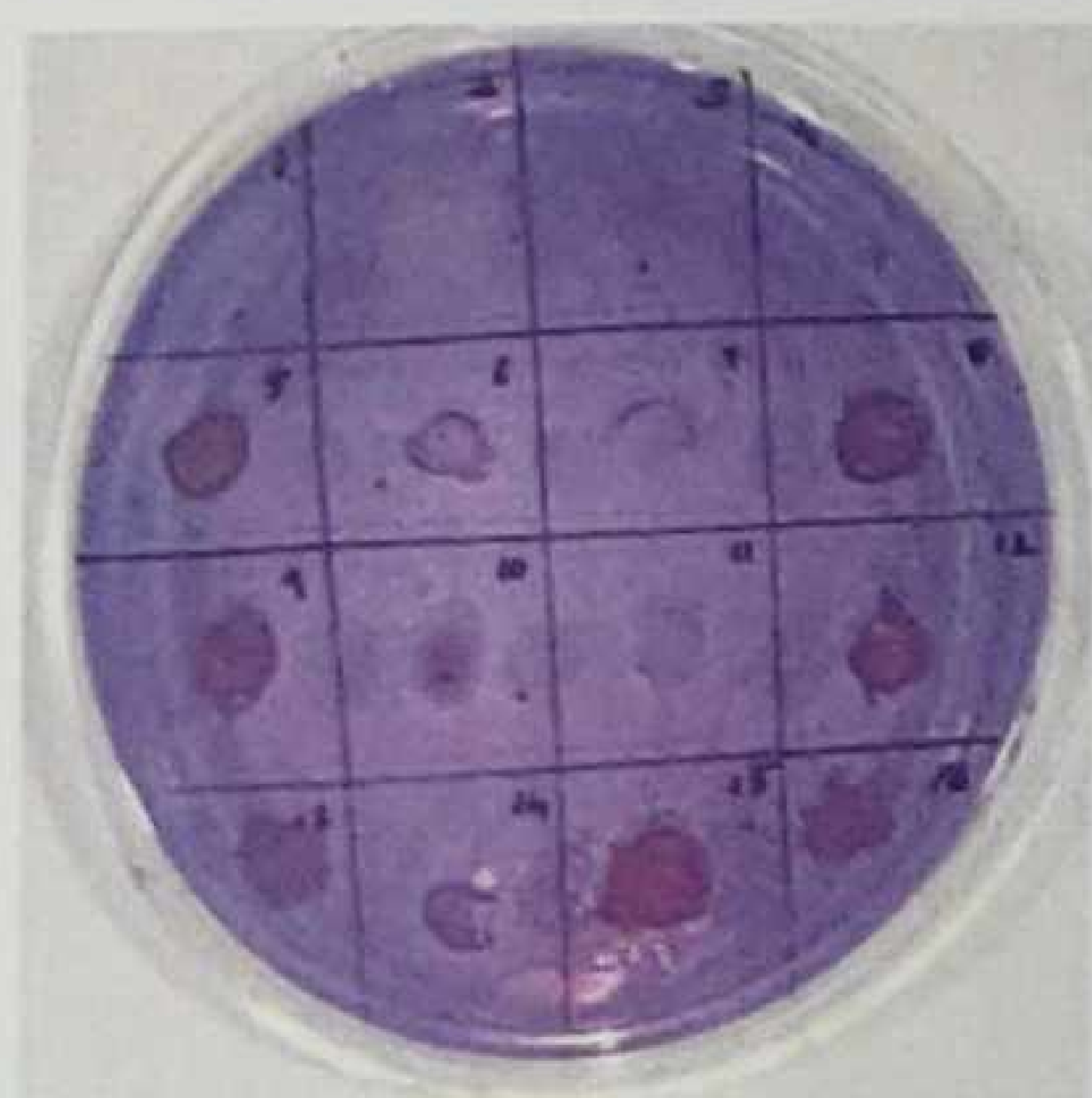
Additionally, 12 isolates demonstrated safranin degradation activity, indicating their potential to degrade this dye commonly used in microscopy and histology applications. Safranin degradation by soil bacteria is essential for mitigating its release into the environment and minimizing its adverse effects on ecosystems (Fig 5.4). (Table 5.5).

Overall, the findings from this study underscore the importance of bacterial-mediated dye degradation in environmental remediation efforts. The ability of soil

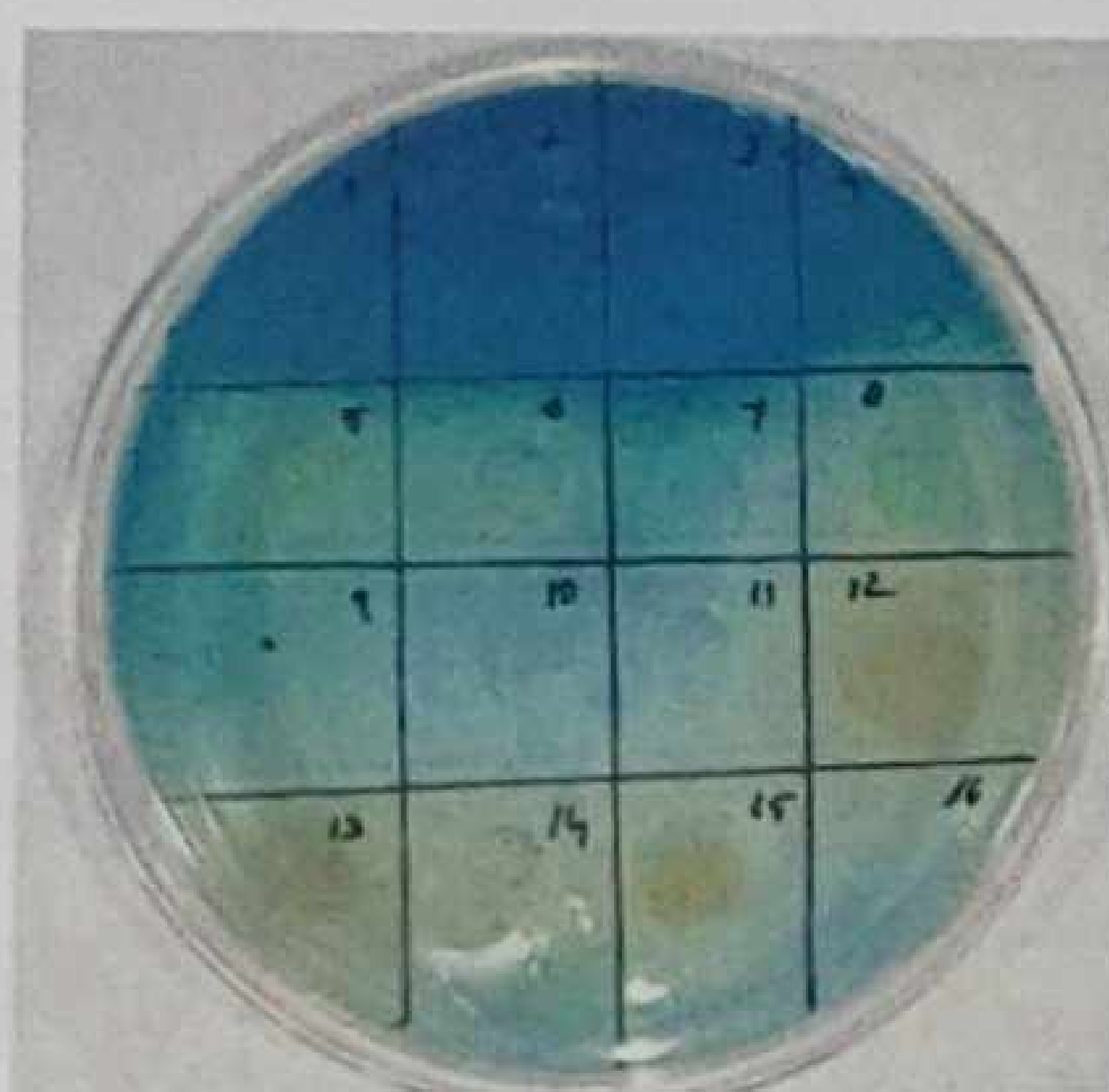
bacteria to degrade a variety of dyes highlights their potential for application in bioremediation strategies aimed at mitigating dye pollution in soil and aquatic environments. Understanding the diversity and distribution of dye-degrading microorganisms within soil ecosystems is essential for the development of sustainable and effective remediation technologies. In previous study by Saravanan, *et al.*, (2015) screened dye degrading bacteria collected from textile effluent.

Table 5.5 Number of isolates showing positive for dye degradation

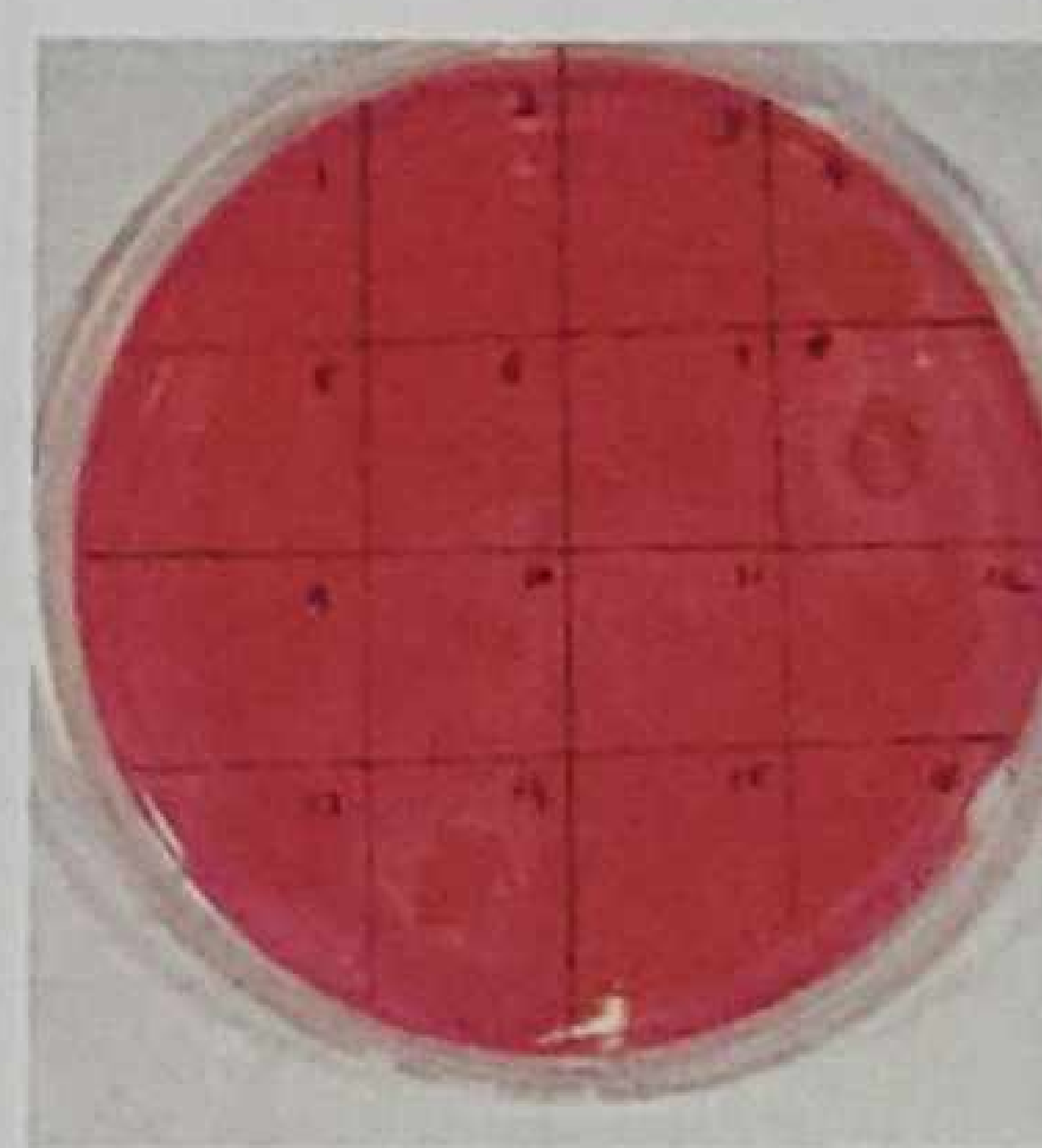
S.NO	Dye	No. of Positive Isolates
1	Crystal violet	26
2	Malachite green	15
3	Safranin	12



(a) Crystal violet



(b) Malachite green



(c) Safranin

Fig 5.4 Dye degradation activity by bacterial isolates

Table 5.6 Isolates report for Dye degradation

Si. NO	Strain No	Screening code	Crystal violet	Malachite green	Safranin
1	THSP1	A1	-	-	-
2	THSP2	A2	+	-	-
3	THSP3	A3	-	-	-
4	THSP4	A4	-	-	-
5	THSP5	B1	+	+	-
6	THSP6	B2	+	+	+
7	THSP7	B3	-	-	-
8	THSP8	B4	-	+	+
9	THSP9	C1	+	-	-
10	THSP10	C2	+	-	-
11	THSP11	C3	-	-	-
12	THSP12	C4	+	+	+
13	THSP13	D1	-	+	-
14	THSP14	D2	-	-	+
15	THSP15	D3	+	+	-
16	THSP16	D4	-	-	-
17	THSP17	A1	-	-	-
18	THSP18	A2	-	-	+
19	THSP19	A3	-	-	-
20	THSP20	A4	-	-	-
21	THSP21	B1	-	-	-
22	THSP22	B2	-	-	-
23	THSP23	B3	-	-	-
24	THSP24	B4	+	+	-
25	SEM25	C1	+	+	+
26	SEM26	C2	+	+	+
27	SEM27	C3	-	-	-
28	SEM28	C4	-	-	-
29	SEM29	D1	-	-	+

30	SEM30	D2	-	-	-
31	SEM31	D3	-	-	-
32	SEM32	D4	-	-	-
33	SEM33	A1	-	-	-
34	SEM34	A2	+	-	-
35	SEM35	A3	+	-	-
36	SEM36	A4	-	-	-
37	M37	B1	+	-	-
38	M38	B2	+	-	-
39	M39	B3	+	-	-
40	M40	B4	+	-	-
41	M41	C1	-	-	-
42	M42	C2	+	+	+
43	M43	C3	-	-	-
44	M44	C4	+	-	-
45	M45	D1	-	-	-
46	M46	D2	-	-	-
47	M47	D3	+	+	-
48	M48	D4	+	-	-
49	M49	A1	-	-	-
50	TR50	A2	-	-	-
51	TR51	A3	+	-	-
52	TR52	A4	+	-	-
53	TR53	B1	+	-	-
54	TR54	B2	+	-	-
55	TR55	B3	+	-	-
56	TR56	B4	-	-	-
57	TR57	C1	-	-	-
58	TR58	C2	+	-	-
59	TR59	C3	+	-	-
60	TR60	C4	+	-	-
61	TR61	D1	-	-	-
62	TR62	D2	-	-	-

63	TR63	D3	+	-	-
64	TR64	D4	+	-	-
65	TR65	A1	-	+	-
66	TR66	A2	-	+	-
67	TR67	A3	+	+	-
68	TR68	A4	+	-	-
69	THS69	B1	-	-	-
70	THS70	B2	-	-	+
71	THS71	B3	-	-	+
72	THS72	B4	-	-	+
73	THS73	C1	-	-	+
74	THS74	C2	-	-	-
75	THS75	C3	-	-	-

5.5. DETECTION OF ANTI MICROBIAL ACTIVITIES OF SOIL BACTERIA

In the antimicrobial screening conducted as part of this study, all 75 bacterial isolates demonstrated one or more antimicrobial activities, indicative of the vast reservoir of antimicrobial compounds produced by soil bacteria. This comprehensive screening provides valuable insights into the potential of soil microorganisms as a source of novel antimicrobial agents for combating infectious diseases and antibiotic resistance.

The antimicrobial screening involved assessing the inhibitory activity of bacterial isolates against a panel of pathogenic microorganisms. Through meticulous experimental procedures and bioassays, the researchers systematically evaluated the antimicrobial efficacy of the soil bacterial isolates.

The results revealed a diverse array of antimicrobial activities exhibited by the bacterial isolates, targeting a range of pathogenic bacteria and fungi. The positive outcomes of the antimicrobial screening were documented in Table 5.7 and illustrated in Figure 5.5, providing a comprehensive overview of the antimicrobial profiles of the soil bacterial isolates.

The antimicrobial activities observed in this study encompassed inhibition of both Gram-positive and Gram-negative bacteria. This broad-spectrum antimicrobial activity underscores the potential of soil bacteria as a source of antimicrobial agents with diverse mechanisms of action.

Furthermore, the antimicrobial screening identified isolates exhibiting potent antimicrobial activities against clinically relevant pathogens, highlighting their potential for therapeutic applications. The discovery of novel antimicrobial compounds from soil bacteria holds promise for addressing the escalating threat of antibiotic resistance and the urgent need for new antimicrobial therapies.

Overall, the findings from this study underscore the importance of exploring soil microbial communities as a source of natural antimicrobial agents. By elucidating the antimicrobial potential of soil bacteria, novel compounds with therapeutic efficacy against infectious diseases can be discovered, paving the way for the development of innovative antimicrobial strategies and

pharmaceutical interventions. In previous study by Mirac Yilmaz, *et al.*, (2006) screened antimicrobial activities of some bacillus spp. Strains isolated from the soil.

Table 5.7 Number of isolates showing positive for antimicrobial activity

S.NO	Test organisms	NO. of Positive isolates
1	Vibrio cholerae	8
2	Aeromonas	6
3	Staphylococcus aureus	4
4	Klebsiella	2
5	Candida	3

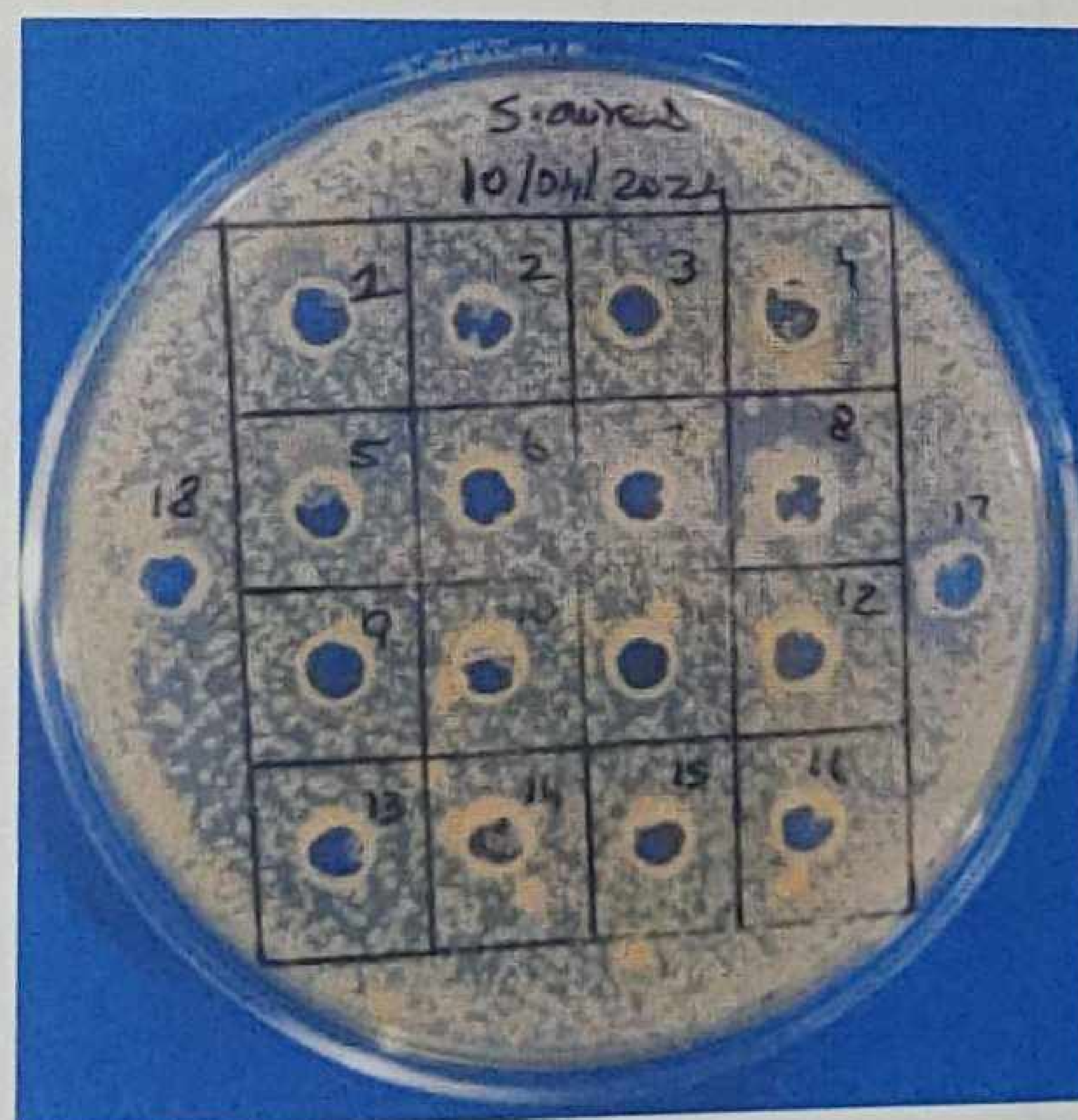


Fig 5.5 Antimicrobial activity by bacterial isolates

Table 5.8 Isolates report for enzymes

Si. NO	Strain No	Screening code	V.Cholerae	Aeromonas	S.Aureus	Klebsiella	Candida
1	THSP1	A1	-	-	-	-	-
2	THSP2	A2	+	-	-	-	-
3	THSP3	A3	-	-	-	-	-
4	THSP4	A4	-	-	-	-	-
5	THSP5	B1	-	-	-	-	-
6	THSP6	B2	+	-	-	-	-
7	THSP7	B3	-	-	-	-	-
8	THSP8	B4	-	-	-	+	-
9	THSP9	C1	-	-	-	-	-
10	THSP10	C2	-	-	-	-	-
11	THSP11	C3	-	-	-	-	-
12	THSP12	C4	-	-	-	-	-
13	THSP13	D1	-	-	-	-	+
14	THSP14	D2	-	-	-	-	-
15	THSP15	D3	+	-	-	-	-
16	THSP16	D4	-	-	-	-	-
17	THSP17	A1	-	-	-	-	-
18	THSP18	A2	-	-	-	-	-
19	THSP19	A3	-	-	-	-	-
20	THSP20	A4	-	-	-	-	-
21	THSP21	B1	-	-	-	-	-
22	THSP22	B2	-	-	-	-	-
23	THSP23	B3	-	-	-	+	-
24	THSP24	B4	-	-	-	-	-
25	SEM25	C1	+	-	-	-	-
26	SEM26	C2	-	-	-	-	-
27	SEM27	C3	-	-	-	-	-
28	SEM28	C4	-	-	-	-	-
29	SEM29	D1	-	-	-	-	-
30	SEM30	D2	-	-	-	-	-
31	SEM31	D3	-	-	-	-	-

32	SEM32	D4	+	-	-	-	-
33	SEM33	A1	-	-	-	-	-
34	SEM34	A2	-	-	-	-	+
35	SEM35	A3	+	-	-	-	-
36	SEM36	A4	+	-	-	-	-
37	M37	B1	+	-	-	-	-
38	M38	B2	-	-	+	-	-
39	M39	B3	-	+	+	-	-
40	M40	B4	-	+	-	-	-
41	M41	C1	-	+	-	-	-
42	M42	C2	-	+	+	-	-
43	M43	C3	-	+	+	-	-
44	M44	C4	-	+	-	-	-
45	M45	D1	-	-	-	-	-
46	M46	D2	-	-	-	-	-
47	M47	D3	-	-	-	-	-
48	M48	D4	-	-	-	-	-
49	M49	A1	-	-	-	-	-
50	TR50	A2	-	-	-	-	-
51	TR51	A3	-	-	-	-	-
52	TR52	A4	-	-	-	-	-
53	TR53	B1	-	-	-	-	-
54	TR54	B2	-	-	-	-	-
55	TR55	B3	-	-	-	-	-
56	TR56	B4	-	-	-	-	-
57	TR57	C1	-	-	-	-	-
58	TR58	C2	-	-	-	-	-
59	TR59	C3	-	-	-	-	-
60	TR60	C4	-	-	-	-	-
61	TR61	D1	-	-	-	-	-
62	TR62	D2	-	-	-	-	-
63	TR63	D3	-	-	-	-	-
64	TR64	D4	-	-	-	-	-

65	TR65	A1	-	-	-	-	-
66	TR66	A2	-	-	-	-	-
67	TR67	A3	-	-	-	-	-
68	TR68	A4	-	-	-	-	+
69	THS69	B1	-	-	-	-	-
70	THS70	B2	-	-	-	-	-
71	THS71	B3	-	-	-	-	-
72	THS72	B4	-	-	-	-	-
73	THS73	C1	-	-	-	-	-
74	THS74	C2	-	-	-	-	-
75	THS75	C3	-	-	-	-	-

5.6 IDENTIFICATION OF ACTIVE STRAINS

SUMMARY AND CONCLUSION

Gram staining microscopic observation

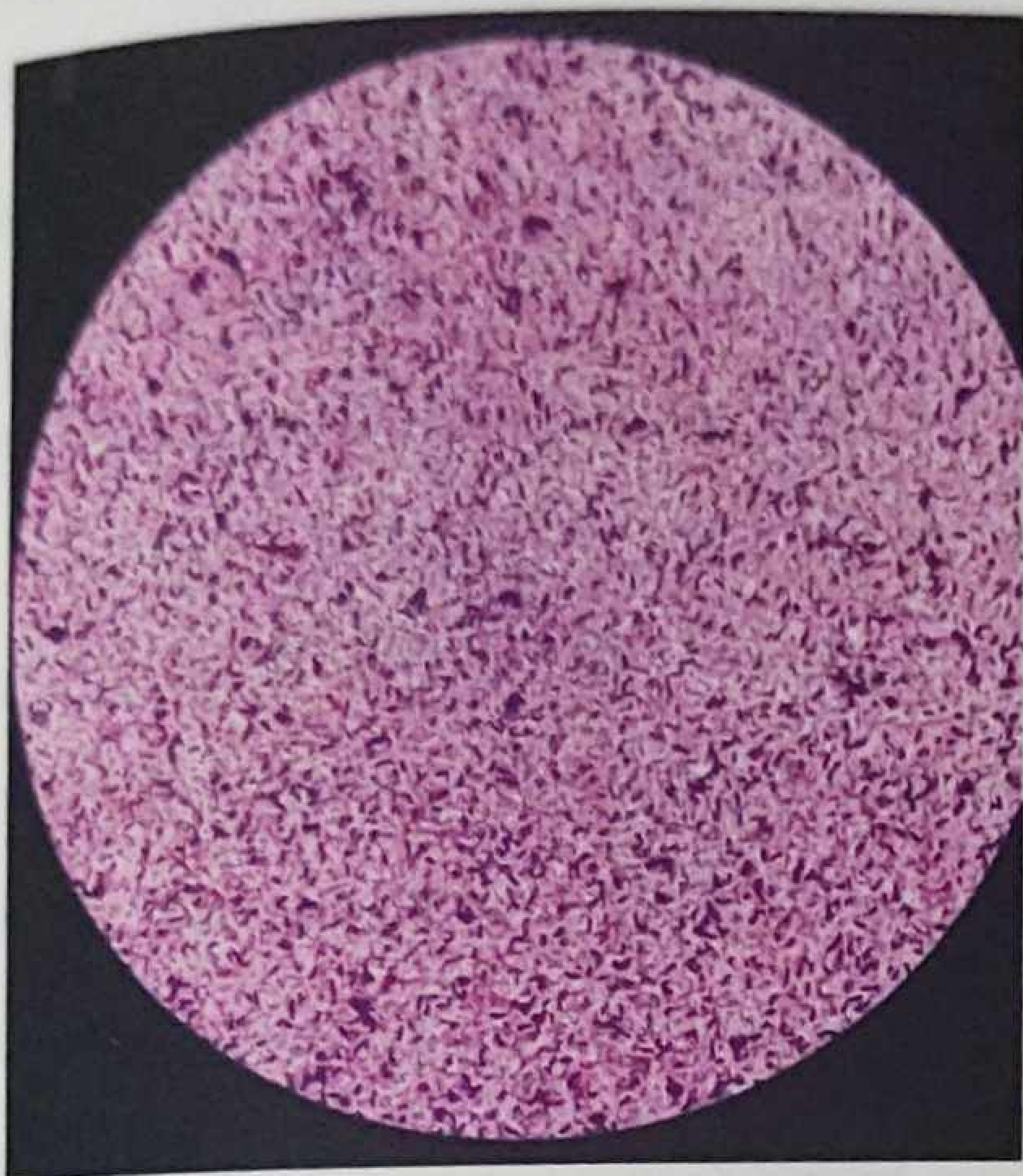


Fig 5.5.1 THSP12

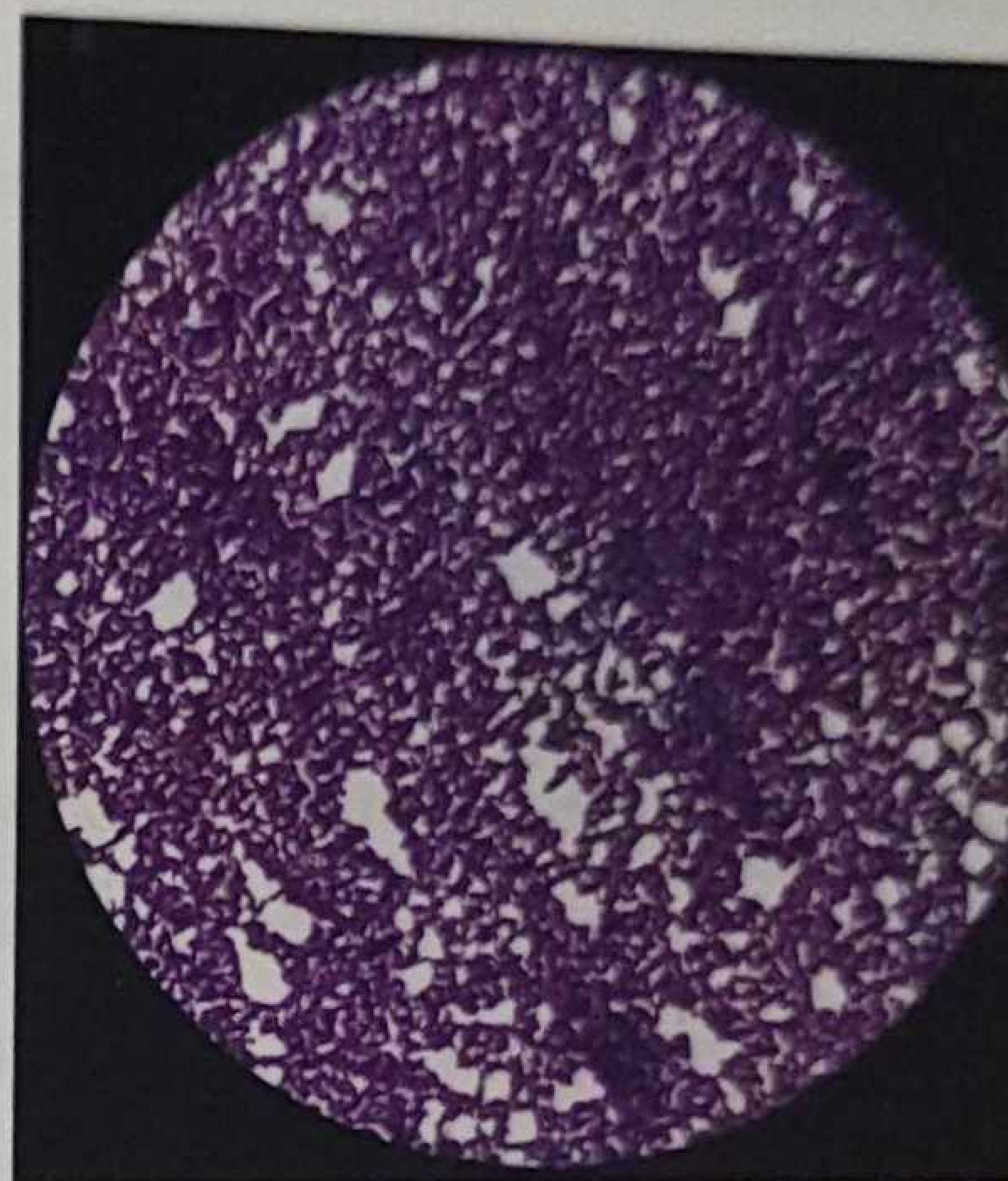


Fig 5.5.2 SEM25

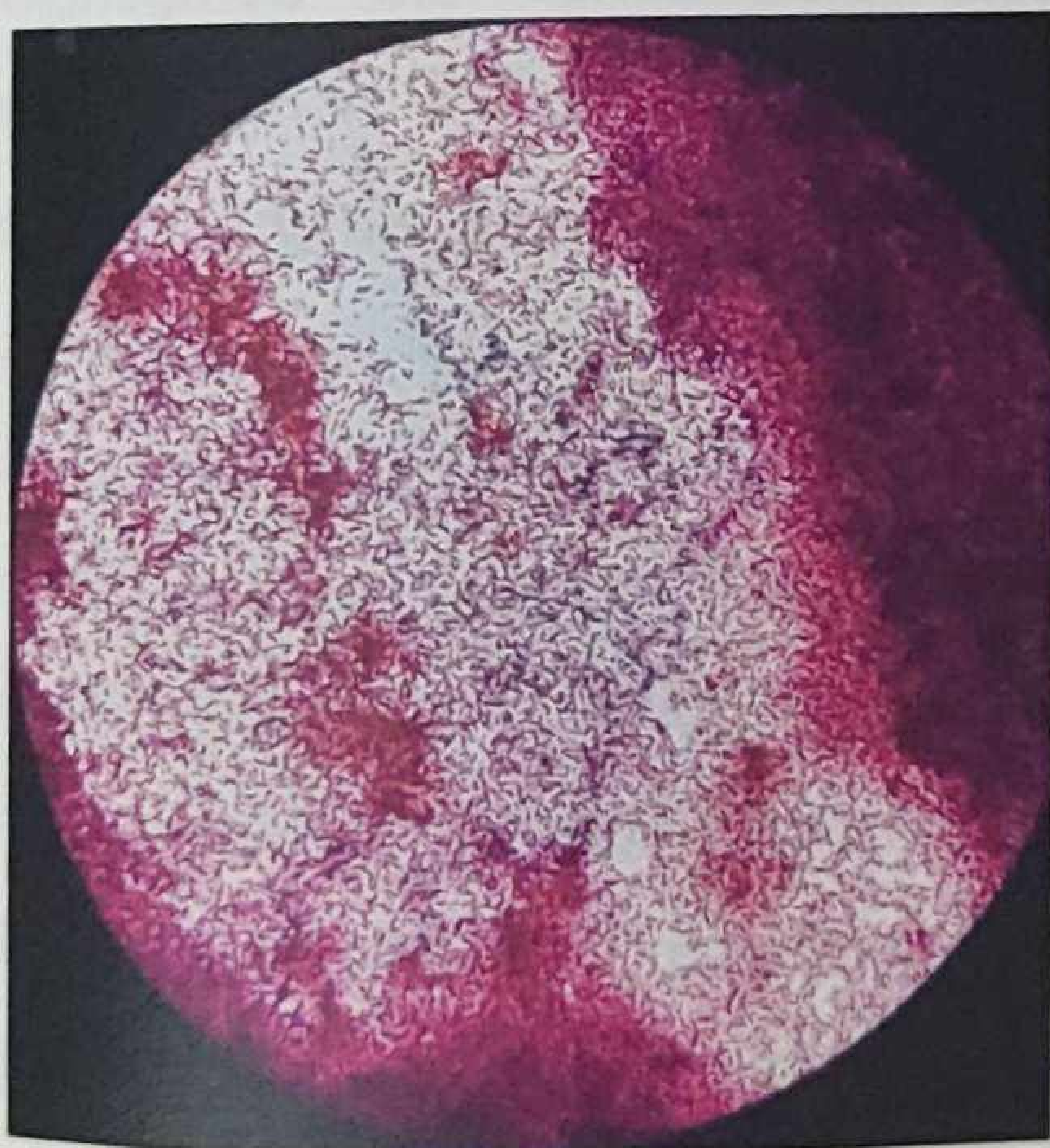


Fig 5.5.3 M40



Fig 5.5.4 M42

These strains are identified as Gram positive and Gram negative rod shaped bacteria. M42 (Fig 5.5.4) is the active strain shows activity in all 3 (Enzymatic degradation, dye degradation, antimicrobial activity)

CHAPTER - 6

SUMMARY AND CONCLUSION

The study encompassed sampling from five distinct environments: Thoothukudi sea, Thoothukudi salt pan, Pichavaram mangrove, Thamirabarani river, and Sembur terrestrial, yielding a total of 75 bacterial isolates. Among these, 7 were from Thoothukudi sea, 24 from Thoothukudi salt pan, 13 from Pichavaram mangrove, 19 from Thamirabarani river, and 12 from Sembur terrestrial. Each of the 75 isolates underwent screening for enzymatic activities, dye degradation capabilities, and antimicrobial potential. Notably, 37 isolates tested positive for amylase, while 15 exhibited protease activity. In terms of dye degradation, 26 isolates displayed crystal violet degradation, 15 showed malachite green degradation, and 12 exhibited safranin degradation. Antimicrobial screening revealed positive results for *Vibrio cholerae*, *Aeromonas*, *Staphylococcus aureus*, *Klebsiella*, and *Candida*, with varying numbers of isolates testing positive for each pathogen. The study's findings highlighted the remarkable diversity of enzymatic, dye degradation, and antimicrobial activities exhibited by bacteria across different soil samples. Noteworthy bacterial isolates included THSP7 and TR60, which showed promising amylase activity, and THS70 and M40, identified as potent sources of protease enzyme. Additionally, isolates such as THSP6, THSP12, SEM25, SEM26, and M47 displayed significant potential for degrading all three tested dyes (malachite green, crystal violet, and safranin). Moreover, bacterial isolates from the Pichavaram mangrove exhibited particularly promising antimicrobial activity compared to those from other sampled environments. These findings underscore the untapped potential of diverse ecosystems as sources of microorganisms with valuable biotechnological applications. The study suggests that further exploration and investigation of these ecosystems could yield insights and resources for various industrial and environmental applications.

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