

GOVERNMENT DEGREE COLLEGE RAMPACHODAVARAM

DEPARTMENT OF ZOOLOGY

ACTIVITIES CONDUCTED IN ACADEMIC YEAR 2024-2025

S.NO	NAME OF THE ACTIVITY	DATE
1	INTERNATIONAL TIGER DAY	29-07-2024
2	GUEST LECTURE ON AQUACULTURE	22-08-2024
3	BLOOD GROUPING	12-09-2024
4	DEWORMING DAY	17-09-2024
5	WORLD ANIMAL DAY	04-10-2024
6	GUEST LECTURE ON ANIMAL DIVERSITY	28-10-2024
7	WORLD FISHERIES DAY	21-11-2024
8	WORLD AIDS DAY	01-12-2024
9	ONE DAY WORKSHOP ON AZOLLA CULTIVATION	07-12-2024
10	FIELD TRIP TO SIFT & CIFE	20-12-2024
11	OPEN DAY -2025	30-01-2025
12	WORLD CANCER DAY	04-02-2025
13	GUEST LECTURE ON GENETIC DISEASES	08-02-2025
14	NATIONAL DEWORMING DAY	10-02-2025
15	AWARENESS PROGRAM ON TB	19-02-2025
16	SUMMER RESEARCH FELLOWSHIP (CSIR – IGIB)	15-04-2025

DEPARTMENT OF ZOOLOGY

July 29, 2024

Report on International Tigers Day



The Department of Zoology organized a programme "International Tigers Day" on 29.07.2024. In this programme we invited Smt.K.Srivani Sub. DFO Rampachodavaram as a chief guest. In this event she spoke about Conservation of wild animals, wildlife protection act's. She also spoke about the importance of tigers in this ecosystem. Students of I,II,III B.Sc Zoology participated in this event.

Guest :

- Smt. K.Srivani, Sub.DFO Rampachodavaram.
- Forest Department Rampachodavaram.

Department Faculty Present:

- Kum. N.Vara Kumari, Lecturer in Zoology
- Sri B.V.V.Satish, Lecturer in Zoology
- Smt. K.Usha Rani Lecturer in Forestry

GOVERNMENT DEGREE COLLEGE RAMPACHODAVARAM

DEPARTMENT OF ZOOLOGY

REPORT ON GUEST LECTURE

Government Degree College ,Rampachodavaram conducted a guest lecture for BSC students on 22-08-2024 ,Dr. K.Veeranjaneyulu , Fisheries Scientist was invited from Krishi Vigyan Kendra Pandirimamidi to give lecture .The topic explained by him was “ Feeding Techniques in Fish Culture ” in his lecture he was also explained Construction of fish farm to all students of I, II, III B.Sc Zoology . The students were participated enthusiastically in this event. Even they come up with few questions.



GOVERNMENT DEGREE COLLEGE RAMPACHODAVARAM

DEPARTMENT OF ZOOLOGY

REPORT ON BLOOD GROUPING

DATE : 12.09.2024

STATION : RAMPACHODAVARM

As per the instructions given by the Principal of Government Degree College Rampachodavaram, Department of Zoology has conducted the Blood group identification test to the all First year students who were admitted in 2024-25 A.Y. Approximately 200 students blood groups were identified in this programme. This programme was conducted in the supervision of N.Vara Kumari Lecturer in Zoology and B.V.V.Satish Lecturer in Zoology. The details of the blood groups were submitted to the Principal of Government Degree College Rampachodavaram.



DEPARTMENT OF ZOOLOGY

SEPTEMBER 17, 2024

Report on Deworming Day



Department of Zoology, Government Degree College Rampachodavaram in collaboration with PHC (Pedda Geddada) distributed Deworming tablets to the students of GDC Rampachodavaram. The medical personnel from PHC Pedda Geddada highlighted the importance of using Deworming tablets "ALBENDAZOLE IP 400 Mg" along with staff and students. These tablets are distributed to 350 students. Kum.N.Vara Kumari Lecturer in Zoology and Sri.B.V.V.Satish Lecturer in zoology encouraged the students to take tablets.

Lecturers Involved :

- Kum. N.Vara Kumari Lecturer in Zoology
- Sri. B.V.V.Satish Lecturer in Zoology
- Medical staff from PHC (Pedda Geddada)

DEPARTMENT OF ZOOLOGY

OCTOBER 04, 2024

Report on World Animal Day



The Department of Zoology has organized and observed "World Animal Day" on 04.10.2024. All staff members from various departments attended this meeting. In this meeting Principal sir explained about the importance of animals in our ecosystem and also he explained about conservation of animals.

- **Department Faculty Present:**

- Kum.N.Vara Kumari, Lecturer in Zoology
- Sri B.V.V.Satish, Lecturer in Zoology
- Kum. G.Sravani, Lecturer in Botany
- Smt. K.Usha Rani, Lecturer in Forestry.

GOVERNMENT DEGREE COLLEGE RAMPACHODAVARAM

DEPARTMENT OF ZOOLOGY

REPORT ON GUEST LECTURE

Government Degree College ,Rampachodavaram conducted a guest lecture for BSC students on 28-10-2024 ,Sri.K.Durga Rao garu , Lecturer in ZOOLOGY was invited from Government College (A) Rajahmundry to give lecture .The topic explained by him was “ ANIMAL DIVERSITY ” in his lecture he was also explained different phylums in invertebrates to all students of I B.Sc Zoology Honours. The students were participated enthusiastically in this event. Even they come up with few questions.



GOVERNMENT DEGREE COLLEGE RAMPACHODAVARAM

DEPARTMENT OF ZOOLOGY

WORLD FISHERIES DAY

DATE: 21-11-2024

STATION: RAMPACHODAVARAM

As per the instructions given by the Principal of GDC, Rampachodavaram, Department of Zoology in collaboration with Krishi Vigyan Kendra (KVK) Pandirimamidi celebrated World Fisheries Day on 21-11-2024. We, B.V.V.Satish Lecturer in Zoology and N.Ashok Lecturer in Zoology with I and II year B.Sc. Zoology Honours students went to KVK, Pandirimamidi.

Dr.K.Veeranjaneyulu sir explained about the fisheries and scope of fisheries. He motivated the students by giving career guidance.

We all went inside to the KVK unit which is around 20 acres. We observed the spawn unit, Bio Flock unit, Azolla unit and Poultry shed and Goat shed. The KVK unit is filled with special Kadaknath, Janapriya, Vanaraja hens, Vusmanabadhi goats, Fodder varieties, Azolla unit, Culture ponds of fisheries. Students from 1st year and 2nd year B.Sc Zoology Honours students acquired knowledge about the fisheries program. All the students are satisfied by visiting KVK Pandirimamidi.



GOVERNMENT DEGREE COLLEGE ,RAMPACHODAVARAM

ACCREDITED BY NAAC WITH "B" GRADE

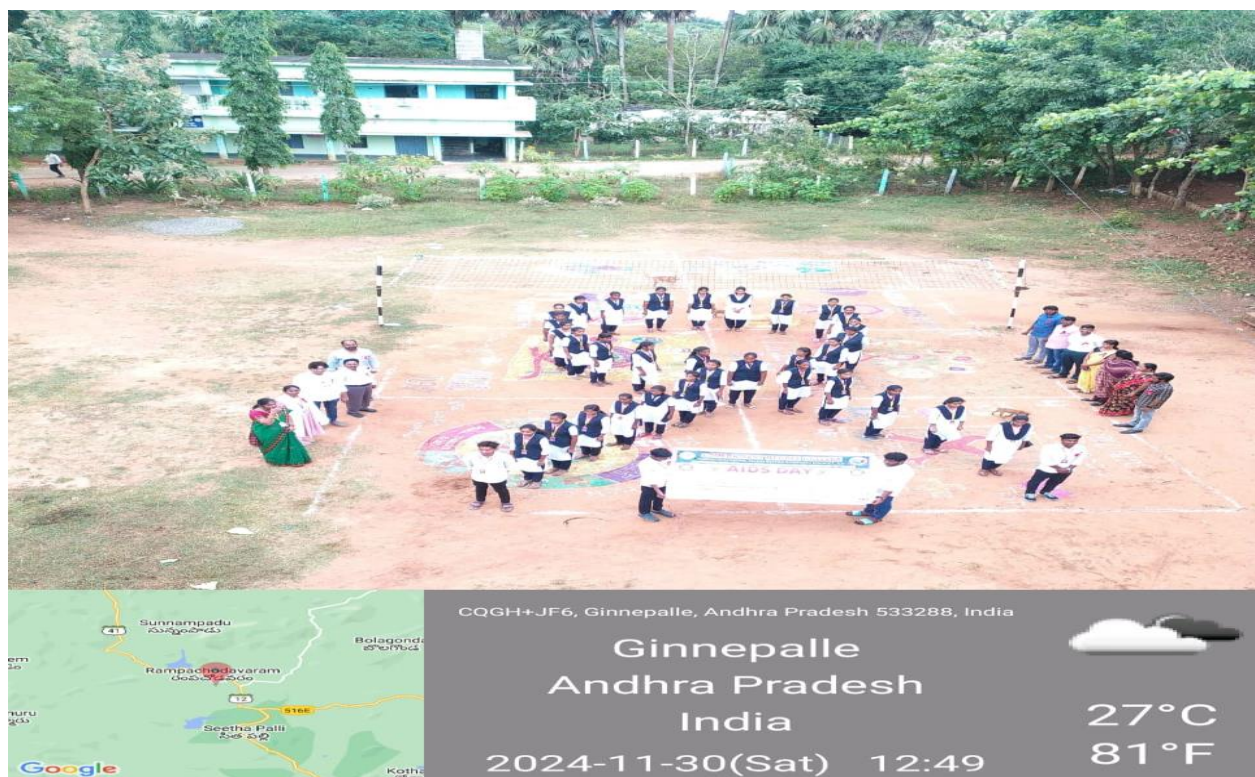
WORLD AIDS DAY -2024 DECEMBER 01

The NSS Unit of Government degree college,Rampachodavaram observed WORLD AIDS DAY. in this connection competitions like Elocution,Poster making presentation and Rangoli were conducted. later on staff and students along with principal Dr.K.Vasudha took a rally and human chain to create awerenss among the public on HIV/AIDS.





On the final day a programme was organized and the principal, Teachers and students spoke about the measures to be taken to curtail this dreadful disease. the principal give away the prizes to the winners of the competitions.





CQGH+JF6, Ginnepalle, Andhra Pradesh 533288, India

Ginnepalle
Andhra Pradesh
India

2024-11-30(Sat) 13:00



27°C

81°F





ప్రభుత్వ డిగ్రీ కళాశాలలో 'వరల్డ్ ఎయిడ్స్ డే'



రంపచోడవరం, మేజర్ న్యూస్ : రంపచోడవరం ప్రభుత్వ డిగ్రీ కళాశాలలో 'వరల్డ్ ఎయిడ్స్ డే' కార్యక్రమాన్ని శుక్రవారం నిర్వహించారు. వరల్డ్ ఎయిడ్స్ డే కార్యక్రమంలో భాగంగా డిగ్రీ కళాశాల ప్రిన్సిపాల్ డాక్టర్ కె.వసుధ ఆధ్వర్యంలో ఎన్ఎస్ఎస్ విభాగం వారు విద్యార్థులకు వక్రత్వం, పోస్టర్ ప్రజెంటేషన్, రంగోలి వంటి పోటీలు నిర్వహించారు. ఈ పోటీల్లో సుమారు 50 మంది విద్యార్థులు పాల్గొన్నారు. ఈ పోటీలకు న్యాయ నిర్ణేతలుగా కళాశాల సీనియర్ అధ్యాపకులు ఎస్.ఆర్.బి.చక్రవర్తి, వైస్ ప్రిన్సిపల్ కె.జీ. వెంకటేష్ వ్యవహరించారు. ఈ కార్యక్రమంలో పలువురు అధ్యాపకులు, సిబ్బంది, విద్యార్థులు పాల్గొన్నారు.



GOVERNMENT DEGREE COLLEGE

RAMPACHODAVARAM, ALLURI SEETHA RAMARAJU DISTRICT, A.P

(Affiliated to Adikavi Nannaya University)

Accredited by NAAC with "B" Grade



BIOLOGICAL DEPARTMENTS

CONDUCTED ONE DAY WORKSHOP ON

AZOLLA CULTIVATION

The Departments of Botany ,Zoology, and Forestry conducted a one Day workshop on Azolla cultivation on **7 December 2024** . Senior scientist Dr.VEERANJANEYULU from krushi vigyan Kendra Rampachodavaram attended the one day workshop as resource person. In the morning session Theoretical session of azolla cultivation was explained to the biological students .In the afternoon session the students were given hands on experience under the guidance of resource person .The students prepared a small unit for azolla cultivation. They choose a small portion in the scooter stand and placed a water proof lining and maintained 5-10 cm of water . Later they filled it with soil , cow dung and single super phosphate .Later azolla was evenly spread on the water surface . The Entire Experience was Enlightening for the students . The principal Dr. k. vasudha Along with the faculty K.Usharani Lecturer in Forestry,G. Sravani lecturer in Botany ,B.V.V. Satish Lecturer in zoology ,N. AshokLecturer in zoology





PREPARING READYMADE AZOLLA BED



డిగ్రీ కళాశాలలో 'అజొల్లా' సాగుపై వర్క్‌షాప్



రంపచోడవరం, మేజర్ న్యూస్ : రంపచోడవరం ప్రభుత్వ డిగ్రీ కళాశాలలో అజొల్లా సాగుపై వర్క్ షాప్ నిర్వహించారు. కళాశాల ప్రిన్సిపాల్ డాక్టర్ కె.వసుధ ఆధ్వర్యంలో అజొల్లా సాగుపై వర్క్‌షాప్ ను కృషి విజ్ఞాన కేంద్రం సీనియర్ మత్స్య శాస్త్రవేత్త డా.కె వీరాంజనేయులు నిర్వహించడం జరిగింది. ఈనందర్భంగా అజొల్లా సాగు, ఉపయోగాలు, పశువులకు మేతగా ఎలా వాడాలి, ఎలా పెంచాలో వివరించారు. అలాగే అజొల్లా యూనిట్‌ను ప్రభుత్వ డిగ్రీ కళాశాలలో బయాలజీ విద్యార్థులుచే నిర్మించడం జరిగింది. ఈ కార్యక్రమంలో డిగ్రీ కళాశాల ప్రిన్సిపాల్ డా.కె.వసుధ, వైస్ ప్రిన్సిపాల్ కె.జివెంకటేష్, జీవశాస్త్ర అధ్యాపకులు బి.వి.వి.సతీష్, కె.ఉషారాణి, జి.శ్రావణి, ఎన్.అశోక్, జీవశాస్త్ర విద్యార్థులు పాల్గొన్నారు.



CENTRAL RESEARCH INSTITUTE FOR DRYLAND AGRICULTURE
KRISHI VIGYNAN KENDRA, (RANGA REDDY Dist.)

AZOLLA PRODUCTION

Dr.SUDHEER.DUSSA
SMS-Vety.SCIENCE

పశువుల మేతగా అజొలా



అజొల్ల గురించి

•అజొల్ల నాచుమొక్కల్లా కనిపించే, నీటిపై తేలియాడే
ఒక కలుపు

మొక్క

•సాధారణంగా అజొల్ల వరి పొలాల్లోనూ, లోతులేని
జలావాసాల్లోనూ పెరుగుతుంది

•అత్యంతవేగంగా వృద్ధి చెందుతుంది.



అజొల్ల

పశుగ్రాసం/మేతగా అజొల్ల

- మాంసకృత్తులు, ఎమైనో అమ్లాలు, విటమిన్లు (ఎ విటమిన్, బి12 విటమిన్ మరియు బెటా-

- కెరోటెన్) ఎదుగుదలకు దోహదకారకాలు, ధాతువులు అయిన కాల్షియం, ఫాస్ఫరస్,

- పోటాషియం, ఫెర్రస్, కాపర్, మాంగనీస్ వంటి వాటితో సమృద్ధమైనది.

- మాంసకృత్తులు 25 - 35 శాతం, ఖనిజాలు 10 - 15 శాతం, ఎమైనో అమ్లాలు, ఖనిజద్రవ్యాలు

- మరియు బయో పాలిమర్స్ 7 - 10 శాతం కలిగిఉండి పోడిభారమాధారమైనది.

- అధికమాంసకృత్తులుండి, కాండభాగం తక్కువగా ఉండటంతో పశువులు త్వరగా జీర్ణం

అజొల్లా తయారీ

- నేలను సమతలం చేసి, కలుపుమొక్కలను ఏరెయ్యాలి
- క్షితిజసమాంతరంగా ఇటుకలను దీర్ఘచతురస్రాకారంలో అమర్చాలి
- ఒక అతినీలలోహితాన్ని స్థిరీకరించిన పోలిథిన్

షీటును దీర్ఘచతురస్రాకారంలో

అమర్చిన ఇటుకల చివరలు కూడా కప్పెట్టబడేలా వాటిమీద
సమాంతరంగా

పరవాలి.

- జల్లించిన 10-15 కిలో గ్రాముల మట్టిని ఈ షీటు పై చల్లాలి
- ఆవుపేడ 2 కిలోగ్రాములు, 30 గ్రాముల సల్ఫర్ ఫాస్పేట్ కలిపిన
ముద్దను 10 లీటర్ల

నీటితో కలిపి పోలిథిన్ షీటు మీద పోయాలి. నీటిమట్టం 10 సెంటీ
మీటర్లకు

చేరేందుకు మరిన్ని నీళ్ళను పోయాలి.

•శుద్ధమైన కణసంవర్ధనం చేసిన అజొల్లవితన ముడి సరుకును,

కిందపరచుకుని ఉన్న అజొల్ల

పాదు పైనున్న మట్టిని, నీటిని సున్నితంగా కదిలించిన తర్వాత చల్లాలి.

అజొల్ల మొక్కలు

నిటారుగా ఎదిగేందుకు అవి భూమిని చీల్చుకుని బయటకు వచ్చిన వెంటనే
తాజానీటిని

చిలకరించాలి.

• ఒక వారంలోఅజొల్ల పాదుమొత్తం పరచుకుని ఒక చిక్కని చాపలా కనిపిస్తుంది.

• శీఘ్ర గతిన అజొల్ల హెచ్చయ్యేందుకు, మరియు రోజువారీ దిగుబడి 500

గ్రాములగా

ఉండేందుకు గాను, సూపర్ సల్ఫేట్ 20 గ్రాములు, ఒక కిలోగ్రాము అవుపేడ
కలిపిన

మిశ్రమాన్ని అయిదురోజుల కొకసారి కలుపుతుండాలి.

• అజొల్ల యొక్క ఖనిజ ద్రవ్యాలను పెంపొందించేందుకు వారానికోతడవ
మెగ్నీషియం, ఇనుము,

రాగి, సల్ఫర్ వంటివి కలిపినసూక్ష్మపోషకమిశ్రమాన్ని కలపవచ్చు.

.తెగుళ్ళు, కీటకాలతో కలుషితమయినప్పుడు పాదును మరలా
సిద్ధంచేసి శుద్ధమైన
కణసంవర్ధనం చేసిన అజొల్లను పాదుకొలపాలి.



బాగా ఎదిగిన
అజొల్ల

అజొల్ల ఉత్పత్తి గుంతలు









పంట కోత

శీఘ్రంగా ఎదుగుతుంది మరియు గుంత ప్రతి 10 - 15 రోజులకొకసారి నిండుతుంది. అక్కడి నుండి ప్రతిరోజూ 500 - 600 గ్రాముల అజొల్లను కోసుకొనవచ్చును.

- పదిహేనవ రోజునుంచి, ప్రతిరోజూ ప్లాస్టిక్ జల్లెడతో గాని, అడుగున చిల్లులున్నతట్టతో

కానీ ఈ పని చెయ్యవచ్చు.

- కోసిన అజొల్లనుంచి ఆవుమూత్రపు వాసనను పోగొట్టేందుకు శుభ్రమైన నీటితో కడగాలి

ప్రత్యామ్నాయ ఉత్పాదకాలు

- తాజాబయోగ్యాస్ మిథినీ కూడావాడొచ్చు
- గుంతను నింపేందుకు స్నానపునీటిని మరియు పశువులపాక నుంచి వచ్చేవ్యర్థపు

నీటిని కూడా గుంతను నింపేందుకు ఉపయోగించవచ్చు.

ఎదుగుదలకు కావాల్సిన పర్యావరణ కారకాలు

- ఉష్ణోగ్రత 20°సి - 28°సి
- వెలుతురు 50% పూర్తి సూర్యకాంతి
- సాపేక్ష తేమ 65 - 80%
- నీరు (గుంతలో నిలవ) 5 - 12 సెంటీమీటర్లు
- పిహెచ్ 4 - 7.5
- అజొల్ల సాగులో గుర్తుంచుకోవాల్సిన సంగతులు
- వల లోపల శుభ్రం చెయ్యటంవల్ల చిన్నచిన్న పిలకలు
బయటపడటానికి

వీలుంటుంది. మరలా వాటిని నీటిగుంటలోకి జారవిడవవచ్చు.

- ఉష్ణోగ్రత 25° సి లోపు ఉండేలా జాగ్రత్త వహించాలి.
- వెలుతురు తీవ్రతను తగ్గించేందుకు అడ్డుగా నీడనిచ్చే వలలు
కాదనవచ్చు.



GOVERNMENT DEGREE COLLEGE

RAMPACHODAVARAM, ALLURI SEETHA RAMARAJU DISTRICT, A.P
(Affiliated to Adikavi Nannaya University)

Accredited by NAAC with "B" Grade



DEPARTMENT OF BIOLOGICAL SCIENCES

Report on the Industrial Field Trip to SIFT and CIFE

Organized by : Government Degree College, Rampachodavaram

Date: 20th December 2024

Introduction

The final-year students of the Biology Department, Government Degree College, Rampachodavaram, undertook an industrial field trip to the State Institute of Fisheries Technology (SIFT) and the Central Institute of Fisheries Education (CIFE) in Kakinada. This visit provided the students with a valuable opportunity to enhance their theoretical knowledge with practical exposure to fisheries management and aquaculture technologies.

Objective of the Visit

The primary objective of the trip was to familiarize students with:

- Modern fisheries management practices.
- Laboratory equipment and their applications.
- Field techniques in aquaculture, including fish breeding and water quality analysis.

Activities and Learning Outcomes

1. Introduction by SIFT and CIFE Officials

- The officers provided an overview of the establishment of fisheries technology in Andhra Pradesh.
- They detailed the infrastructure and facilities available at their institutes.
- The staff actively engaged with students, ensuring a hands-on learning experience.

2. Laboratory Exposure

- Students were introduced to several advanced laboratories, including:
 - Microbiology Lab
 - Feeding Lab
 - Chromatography Lab
 - PCR Analysis Lab
 - Soil Analysis Lab
 - Water Analysis Lab

- Practical demonstrations were conducted to help students understand the functions and importance of the laboratory equipment used in fisheries research and management.

3. Field Visit

- The students visited the CIFE fields where they observed real-world applications of aquaculture practices, including:
 - **Hapas breeding techniques**
 - **Spawning units**
 - **Cultivation of fresh water and marine fishes**
 - **Marine prawn farming**
- Farmers working at the CIFE interacted with the students, sharing their experiences and practices.

Faculty Participation

The field trip was guided and coordinated by the faculty of the Biology Department:

- **B.V.V.Satish (Lecturer in Zoology)**
- **N.Ashok (Lecturer in Zoology)**
- **K.Usharani (Lecturer in Forestry)**
- **G. Sravani (Lecturer in Botany)**

Conclusion

The field trip was a highly enriching experience for the students, offering them a platform to gain hands-on knowledge about aquaculture management, laboratory procedures, and fisheries technology. The students actively participated in practical demonstrations, showing enthusiasm and keen interest in the subject.

Such initiatives bridge the gap between classroom learning and real-world applications, equipping students with the skills and confidence needed for future endeavors in the field of fisheries and aquaculture.

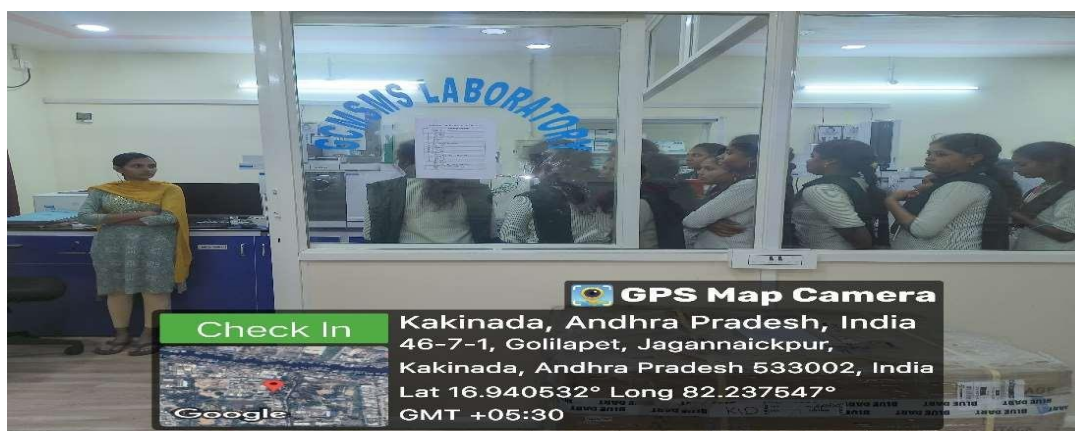




FISH MUSEUM :



GAS CHROMATOGRAPHY MS.MSLAB :



FEEDING LAB :



INTRACTION WITH FDO :





GOVERNMENT DEGREE COLLEGE RAMPACHODAVARAM
DEPARTMENT OF ZOOLOGY
OUT REACH PROGRAM TO INTERMEDIATE STUDENTS

As per the instructions of the Principal the department of Zoology has conducted an outreach program to intermediate students (II Year Bi.P.C) “ OPEN DAY ”.

Students from APTWR (Girls & Boys) attended to this program. Students acquired knowledge about the specimens, slides, live experiments, charts. In this program M.Yedukondalu student from (III B.Sc B.Z.F) along with zoology lecturers performed the live experiments which will be useful to their public practical examinations. He performed the experiments like starch identification, action of salivary amylase on carbohydrates, lipid test, albumin test. After practical session we also gave a chance to the students to perform the experiment on their own. N.Ashok lecturer in Zoology explained the practical exam question paper and identification of specimens and also he gave some tips to the students which are very useful to gain good marks in practical examination. B.V.V.Satish lecturer in Zoology motivated the students to join in GDC RCVM.

An orientation program is also conducted to the students about single major courses. In this program Dr.K.Vasudha Principal GDC Rampachodavaram gave a brief idea about degree education system and also she explained the single major courses offered by GDC RCVM. This is a great program conducted by department of Zoology to grab the opportunity to fulfill the seats in upcoming academic year 2025-2026. Principal K.Vasudha madam appreciated the efforts of the lecturers who involved in this program.

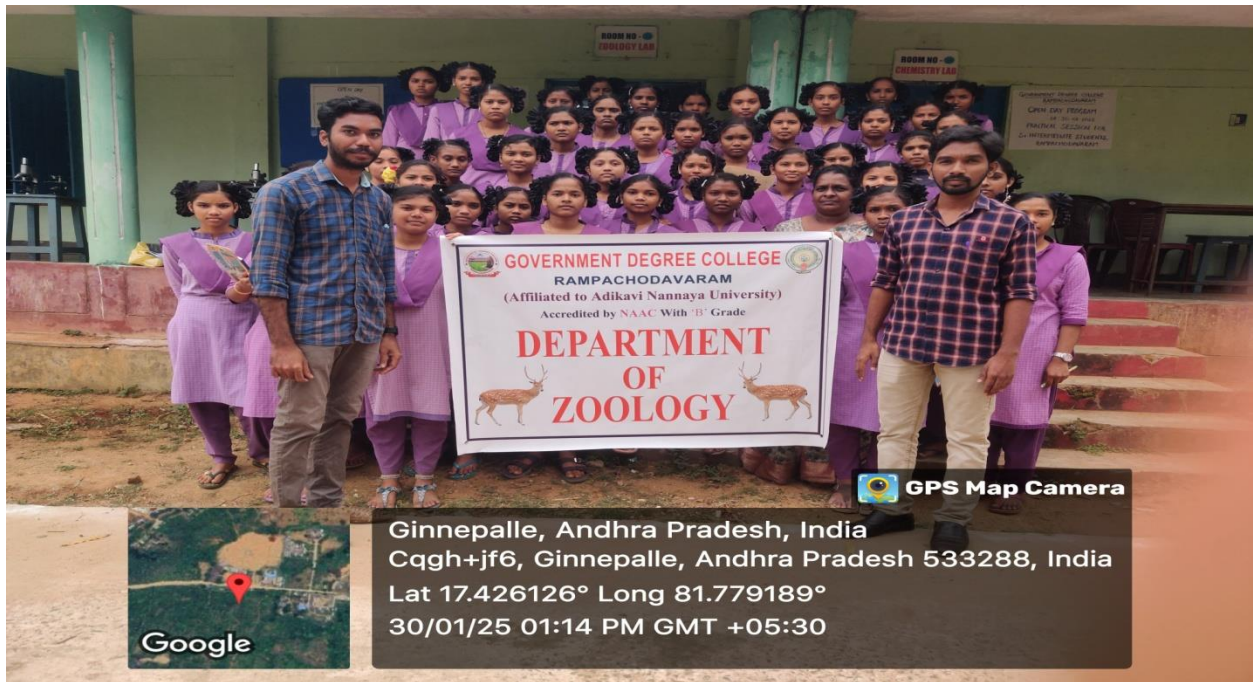
Objectives of the program :

- To fulfill the admissions in A.Y.2025-2026
- To create awareness about single major courses
- To gain attention of intermediate students

Out comes of the program :

- Students gained knowledge about practical examination.
- Students understand about single major courses.
- Students understand the courses offered by GDC RCVM.

APTWR GIRLS JUNIOR COLLEGE RAMPACHODAVARAM

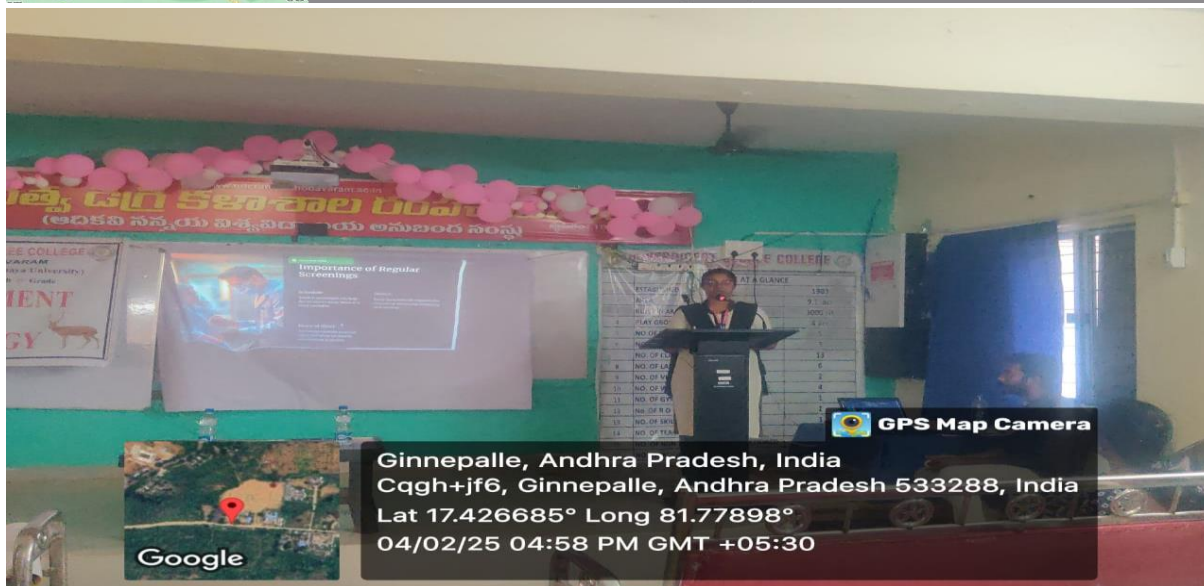


PRACTICAL SESSION FOR STUDENTS



IDENTIFICATION OF SPECIMENS





GUEST LECTURE ON GENETIC DISORDERS, CANCER, AND AUTO IMMUNE DISEASES

STATION : RAMPACHODAVARAM

Dr. K.Vasudha Principal GDC Rcvm addressed the meeting with her valuable words and she expressed her views on cancer and other genetical issues after that Dr. D. Sujesh Kumar sir presented a PPT on the above mentioned issues. Students were happy with his presentation and showed interest in knowing the diseases, causes and precautions.

Later on he shared his valuable experience in the field of research. Students felt happy with his presentation and principal appreciated the lecturer (B.V.V.Satish) for his contribution towards this program.



DEPARTMENT OF ZOOLOGY

FEBRUARY 02, 2025

Report on Deworming Day

Department of Zoology, Government Degree College Rampachodavaram in collaboration with NSS Unit distributed Deworming tablets to the students of GDC Rampachodavaram and Bornagudem school students. K.G. Venkatesh Incharge Principal highlighted the importance of using Deworming tablets “ALBENDAZOLE IP 400 Mg” to staff and students. These tablets are distributed to 350 students. B.V.V.Satish Lecturer in Zoology and D. Ravi Kumar Lecturer in telugu encouraged the students to take tablets.

Lecturers Involved :

- Sri. K.G. Venkatesh (Lecturer in Chemistry)
- Sri. B.V.V.Satish (Lecturer in Zoology)
- Sri. D.Ravi Kumar (Lecturer in Telugu)



GOVERNMENT DEGREE COLLEGE RAMPACHODAVARAM
DEPARTMENT OF ZOOLOGY
REPORT ON TB AWARENESS RALLY

DATE : 19-02-2025

STATION : RAMPACHODAVARAM

Department of Zoology GDC Rampachodavaram and Government Area hospital Rampachodavaram has observed the “TB AWARENESS DAY “on 19-02-2025. We collaborated with Area Hospital and conducted a program with students and created awareness among the society. The program was interesting to the Students from 2nd year Zoology and 3rd B.Sc B.Z.C. & B.Z.F. participated. Doctors from Government Area Hospital explained the symptoms, treatment to the students. Dr. K.Vasudha Principal and B.V.V.Satish lecturer in zoology participated in this program.



GOVERNMENT DEGREE COLLEGE RAMPACHODAVARAM

DEPARTMENT OF ZOOLOGY

REPORT ON CSIR SUMMER RESERCH FELLOWSHIP PROGRAMME -2025

The Department of Zoology encouraged the students to participate in work shops, seminars, PG Entrance exams. M. Yedukondalu (III B.Sc B.Z.F.) applied for the **CSIR Summer Research Fellowship Programme 2025**. Principal Dr. K.Vasudha encouraged the students in all aspects. She gave her valuable suggestions to the student. Later the student was selected for two months summer research programme. Officials from CSIR Institute IGIB contacted the student through E-mail. M. Yedukondalu was selected and the confirmation mail was received. Then immediately principal and staff members were very happy and they congratulated the student and they sent the student to New Delhi for **2 months CSIR Summer Research Fellowship Programme**. M. Yedukondalu reported to the allotted Guide **Dr. Sheetal Gandotra Senior Principal Scientist and Infectious Disease Biology Unit (CSIR – IGIB New Delhi)**. Under the Guidance of Dr. Sheetal Gandotra he completed his project on **Using Macrophage Infection Models to Understand Host-Pathogen Interaction in Mycobacterial Infections**.

NAME OF SRF : M. Yedukondalu

REGISTRATION NO : LFS1831

INSTITUTION WHERE WORKING : CSIR – IGIB, NEW DELHI

DATE OF JOINING : 15/04/2025

DATE OF COMPLETION OF THE PROJECT : 15/06/2025

NAME OF THE GUIDE : Dr. SHEETAL GANDOTRA

PROJECT NAME : Using Macrophage Infection Models to Understand Host-Pathogen Interaction in Mycobacterial Infections.



सी.एस.आई.आर., जीनोमिकी और समवेत जीवविज्ञान संस्थान
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद्, भारत सरकार)
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CSIR-Institute of Genomics & Integrative Biology
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Dr. Chetana Sachidanandan
Head, HR Division
E-mail: chetana@igib.res.in

CSIR-IGIB/HR/T-GR/AL#1591

Date: 15-04-2025

Mr Yedukondlu Marmam

Sub: Trainee at CSIR-IGIB - Delhi

I am happy to inform you that your application for **Trainee** has been accepted and you will be supervised by

Dr. Sheetal Gandotra for the period from **15-Apr-25 to 15-Jun-25** Extension will be granted only in exceptional cases.

The work carried out by the trainee shall belong to CSIR-IGIB only and CSIR-IGIB shall publish it, if so required. It is mandatory to get signature on NDA (Non Disclosure Agreement) from your supervisor at CSIR-IGIB, prior to joining the institute for training.

At the time of joining, please report to your guide (contact details on website), fill the form attached and get it signed by supervisor and submit to HR at IGIB. Please note that change of guide is not permissible.

After completion of training, certificate will be issued upon submission of report for above mentioned duration.

A soft copy of the progress report along with your NDA (scan copy) is required to be submitted in HRD with clear mention of title of your report.

Note: . Trainee fee is waived off.

Sincerely yours,

Head, HR Division

This is computer generated letter, no signature required

Tel. : 91-11-27667602, 27666156-157, 27667439 • Fax : 91-11-27667471
Telefax : 91-11-27662407-08, 27662099 • E-mail : info@igib.res.in • Website : www.igib.res.in

PHOTOGRAPHS WHILE DOING WORK IN IGIB



PHOTOGRAPH WITH Ph.D SCHLOARS AND IGIB TEAM IN NEW DELHI



PROJECT REPORT

IAS-NAS-INSA

Summer Research Fellowship Programme-2025



USING MACROPHAGE INFECTION MODELS TO UNDERSTAND HOST-PATHOGEN INTERACTION IN MYCOBACTERIAL INFECTIONS

M. Yedukondalu

Government Degree College, Rampachodavaram
Affiliated to Adikavi Nannaya University, Rajamahendravaram



GOVERNMENT DEGREE COLLEGE RAMPACHODAVARAM

(Affiliated to Adikavi Nannaya University)

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ISO9001 : 2015, ISO14001 : 2015, ISO50001 : 2018, Certified Organization

Accredited by NAAC with "B" Grade



Under the guidance of:

Dr. Sheetal Gandotra

Senior Principal Scientist and
Infectious Disease Biology Unit
CSIR- Institute of Genomics and Integrative Biology
South Campus, Mathura Road, New Delhi- 110025



IGIB
INSTITUTE OF GENOMICS
& INTEGRATIVE BIOLOGY
Genomics Knowledge Partner

NAME OF SRF : M.Yedukondalu
REGISTRATION NO : LFS1831
INSTITUTE WHERE WORKING : CSIR-IGIB, NEW DELHI
DATE OF JOINING : 15/04/2025
DATE OF COMPLETION OF THE PROJECT : 15/06/2025
NAME OF THE GUIDE : Dr. SHEETAL GANDOTRA

PROJECT NAME : **Using Macrophage infection models to understand Host–pathogen interaction in Mycobacterial infection**



Dr. Sheetal Gandotra
Senior Principal Scientist
Infectious Disease Biology Unit
CSIR- Institute of Genomics and Integrative Biology
South Campus, Mathura Road, New Delhi- 110025

M. Yedukondalu
LFS1831



जीनोमिकी और समवेत जीव विज्ञान संस्थान
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद)
विश्वविद्यालय परिसर, माल रोड, दिल्ली-११०००७
Institute of Genomics & Integrative Biology
(COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH)
DELHI UNIVERSITY CAMPUS
MALL ROAD, DELHI-110007, INDIA

CERTIFICATE

This is to certify that **M. Yedukondalu** bearing Registration No. **LFS1831** has completed a research internship under the supervision of **Dr. Sheetal Gandotra**, Senior Principal Scientist, at the **Infectious Disease Biology Unit, CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB)**. The internship was carried out as part of the project entitled: **“Using macrophage infection models to understand host-pathogen interaction in mycobacterial infections.”** During the tenure, the student actively participated in experimental work, data analysis, and discussions relevant to the project and demonstrated scientific rigor and enthusiasm. The work exhibited a commendable dedication, curiosity, and collaborative spirit.

Duration: April 15th, 2025 – June 15th, 2025.

Place: New Delhi

Date:

Dr. Sheetal Gandotra
Senior Principal Scientist
CSIR-IGIB

(Official Seal/Signature)

DECLARATION

I, M.Yedukondalu, declare that the work presented in this report entitled: “Using macrophage infection models to understand host-pathogen interaction in mycobacterial infections. I have carried it out as part of my research internship under the supervision of **Dr. Sheetal Gandotra, Senior Principal Scientist and Infectious Disease Biology Unit, CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB)**. I further declare that this work is original and has not been submitted elsewhere for any degree, diploma, or fellowship award. All sources of information have been duly acknowledged.

Place: Rampachodavaram.

M.Yedukondalu.

Date :

Signature of the Student

ACKNOWLEDGMENT

The journey of writing this Report has been my first profound experience with scientific research. While I have strived to put forth my best efforts, I am deeply humbled and immensely grateful for the invaluable guidance, support, and encouragement I have received from so many incredible individuals. Their generosity, patience, and belief in me have played a crucial role in the successful completion of this work.

I would like to express my profound gratitude to **Dr. Sheetal Gandotra**, Senior Principal Scientist and Infectious Disease Biology Unit, CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB), for her exceptional mentorship, invaluable guidance, and constant support throughout this research. Her scientific insight and encouragement have played a pivotal role in shaping this work.

I extend my sincere gratitude to **Ms. Muskan Goel** for her dedicated supervision, patience, and expert guidance, which significantly contributed to my technical and conceptual development during this project.

I am also thankful to the members of the laboratory—**Neetu, Vaishnavi, Priya, Manya, Anwesha, Milin, Arpita, Rakesh, Sachin, Rabia, Adithya, Amit, and Ritika** for their assistance, collaboration, and for fostering a positive and intellectually stimulating research environment.

I gratefully acknowledge the **CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB)** for providing the necessary infrastructure, resources, and institutional support essential for the successful completion of this work.

Lastly, I am deeply indebted to my **family and friends** for their unwavering support, understanding, and encouragement throughout this journey. Their presence has been a constant source of strength and motivation.

With immense gratitude and respect,

M.Yedukondalu.

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Result and Discussion.....	19
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Key words:

Mycobacterium tuberculosis

M. marinum

THP – 1 cell line

Monocytes

Macrophages

RPMI-1640 - Roswell Park Memorial Institute 1640

Tween 80

Amikacin

SYTOX Green

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease that mainly affects the lungs, termed as pulmonary tuberculosis, but can also affect other organs like the skin, lymph nodes, heart, spine, Central Nervous System, etc, termed as extra-pulmonary tuberculosis. TB remains one of the most serious health threats worldwide, with 10.8 million active cases and 1.15 million deaths in 2023 due to TB. According to the Global TB Report 2024 by WHO, TB has become the leading cause of death from a single infectious agent, replacing COVID-19 worldwide (Global TB Report 2024, WHO). The causative agent of tuberculosis is *Mycobacterium tuberculosis* (*Mtb*). When an actively infected person coughs, sneezes, or talks, the water droplets containing the bacteria are released into the environment. These droplets can enter any healthy person's body through the nose or mouth and can reach the alveoli. Alveolar macrophages engulf the bacteria and simultaneously secrete cytokines to recruit other immune cells like neutrophils, dendritic cells, natural killer (NK) cells, T lymphocytes, and B lymphocytes. All these cells aggregate to form a solid structure called a granuloma, which is the hallmark characteristic of TB. As the disease progresses, the granuloma ruptures and thousands of bacteria are released into the airway for further transmission (Rahlwes *et. al.*, 2023).

Studying host–pathogen interactions is essential for developing effective therapeutic and preventive strategies. In humans, immune cells, particularly macrophages, not only serve as the first line of defence but also represent the primary intracellular niche exploited by mycobacteria. In recent years, in vitro macrophage infection models have become essential for investigating the molecular and cellular dynamics of mycobacterial infections. Cell lines such as THP-1, RAW 264.7, or primary macrophages provide controlled systems to study bacterial entry, survival, replication, and host responses.

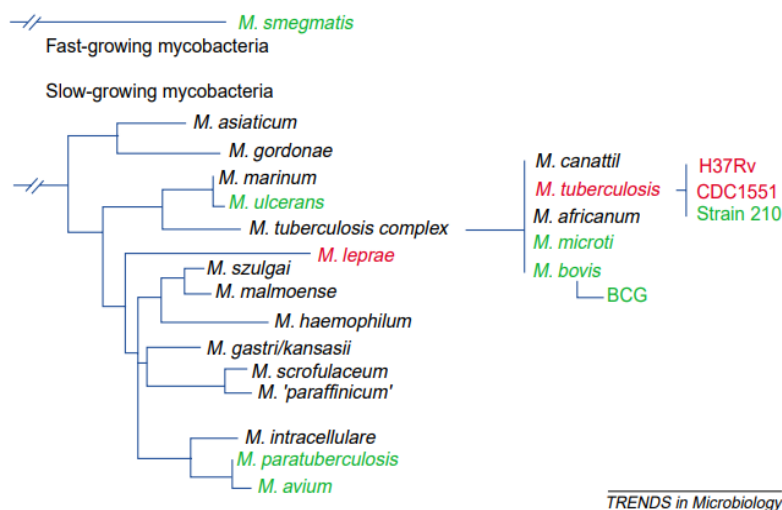


Figure 1: Evolution of Mycobacterial pathogenicity (Brosch *et. al.*, 2001)

MYCOBACTERIA

Mycobacterium tuberculosis belongs to the genus *Mycobacterium*, characterized by a thick, lipid-rich cell wall containing mycolic acids, contributing to their resistance. Over 190 species have been identified, most of which are free-living and inhabit diverse niches. Slow-growing species include *M. asiaticum*, *M. goodii*, *M. marinum*, and *M. tuberculosis*, while *M. smegmatis* is a fast-growing species (J.B. Payeur, 2014, Encyclopedia of food microbiology). *M. marinum* and *Mtb* have more than 99.3% genome similarity and share major virulence factors. *M. marinum* has a faster doubling time (6-8 hours) as compared to *Mtb*, which has a doubling time of 24-30 hours. Where a BSL3 facility and special training are needed to handle *M. tuberculosis*, *M. marinum* can be handled at a BSL2 safety level. *M. marinum* is a non-tuberculous mycobacterium. It causes a tuberculosis-like illness in fish. In humans, when injured skin is exposed to an aqueous environment contaminated with *M. marinum*, infection occurs (Chen *et. al.*, 2017; Brosch *et. al.*, 2001).

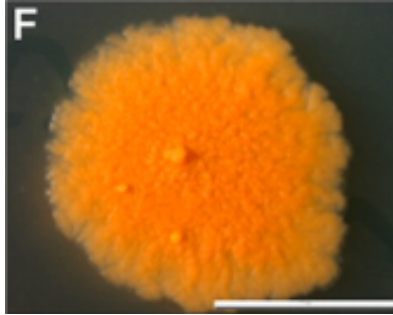


Figure 2: *Mycobacterium marinum* (Julian *et. al.*, 2010)

THP-1 CELLS AS A MODEL OF INFECTION:

Monocytes, derived from myeloid progenitors during haematopoiesis, are the largest type of white blood cells and differentiate into macrophages upon entering tissues. Macrophages, including resting M0, pro-inflammatory M1, and anti-inflammatory M2 subsets, play essential roles in immune defence, tissue monitoring, and homeostasis. M0 macrophages express markers like CD14 and CD68 and polarize into M1 or M2 in response to cytokines. M1 macrophages, activated by IFN- γ and LPS, produce pro-inflammatory cytokines such as TNF- α and IL-1 β , while M2 macrophages, induced by IL-4, IL-10, or IL-13, promote tissue repair and secrete IL-10 and TGF- β , expressing CD163 and CD206 (Clare Brown, 2020, macrophages in microbiology).

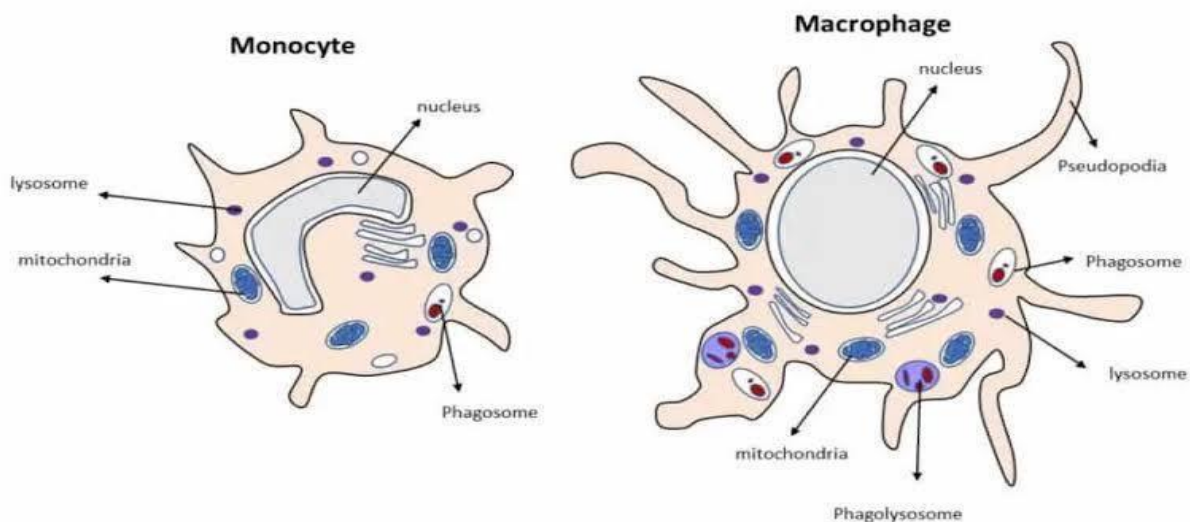


Figure 3: Showing the Monocytic stage and Macrophage stage (Clare Brown, 2020)

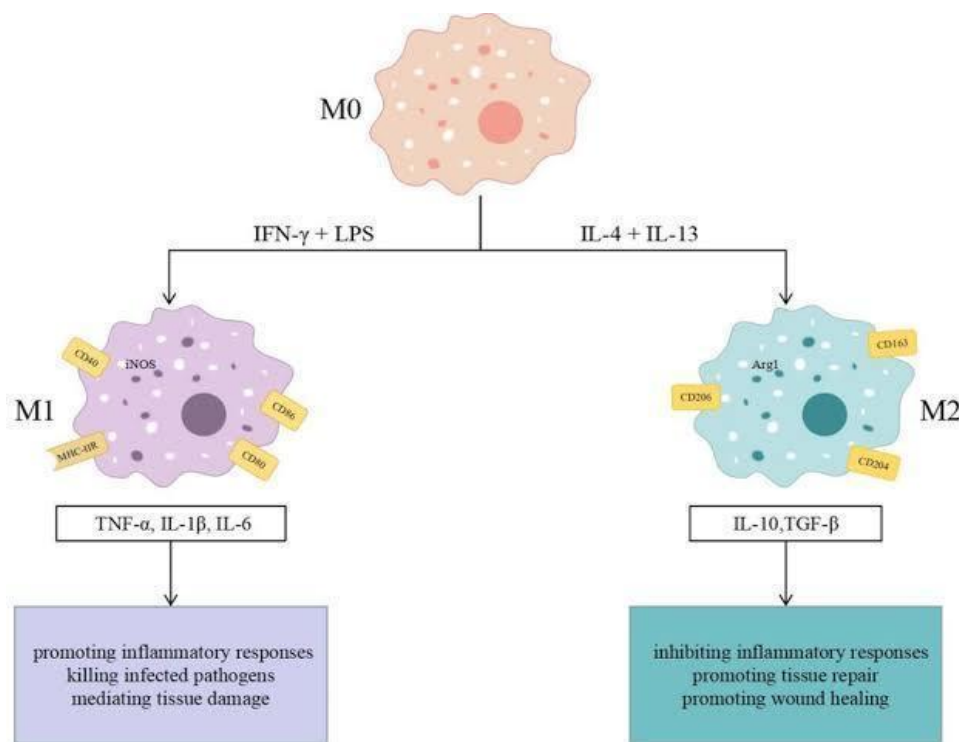


Figure-4 : Differentiation of M0 macrophage into M1 and M2 Macrophages (Xia., et al, 2023)

The human leukemic cell line THP-1 is widely used as an in vitro model to study host–pathogen interactions. Established in 1980, THP-1 cells were derived from the peripheral blood of a patient with acute monocytic leukaemia. THP-1 cells can be differentiated into macrophage-like cells using phorbol 12-myristate 13-acetate (PMA), with optimal differentiation typically achieved after 48 hours of incubation at minimal PMA concentrations. Following differentiation, a 24-hour resting period in PMA-free media is recommended to enhance macrophage marker expression. Differentiated THP-1 macrophages exhibit increased adherence, phagocytic capacity, and expression of surface markers such as CD14, CD36, and TLR-2 (Xia et al., 2023).

Aim of the study:

Establishing a Macrophage model system to perform various screens to investigate host-pathogen interactions during mycobacterial infection.

Objectives:

- 1) Generation of Teal and Blue fluorescent protein expressing *M. marinum* cells.
 - a) Isolation of pTEC17 (mTFP1) and pTEC18 (EBFP2) plasmids using the Qiagen Mini Prep kit.
 - b) Preparation of *M. marinum* electrocompetent cells.
 - c) Electroporation of pTEC17 and pTEC18 plasmids in *M. marinum* electrocompetent cells.
- 2) Growth curve analysis of Teal and Blue fluorescent protein expressing *M. marinum*.

Materials Required: Glycerol stocks of pTEC17, pTEC18 plasmids, and *M. marinum* (Wild type).

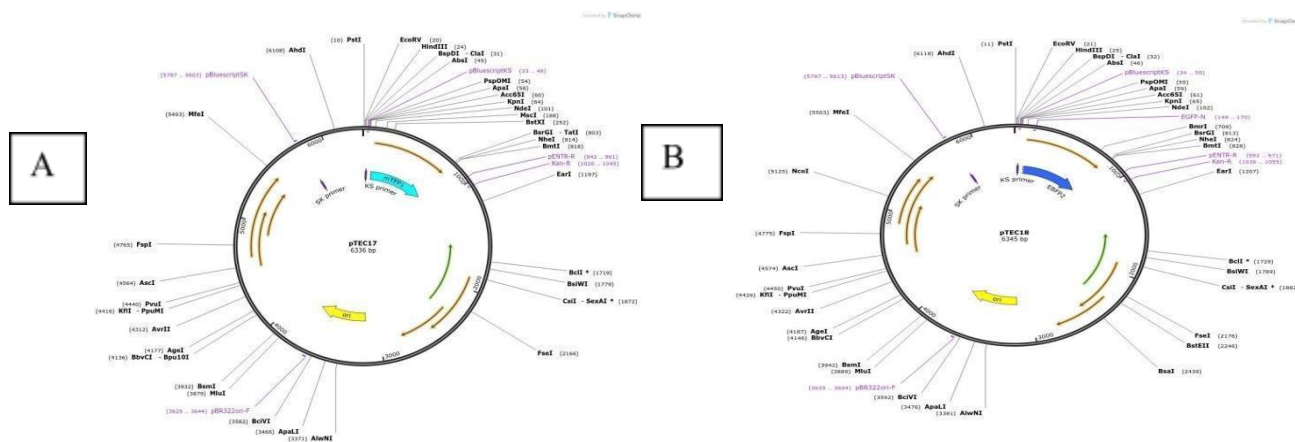


Figure 5: Plasmid map of pTEC17 plasmid (A) and pTEC18 (B)

Methodology:

1. Plasmids, Bacterial Strains, and Culture

Mycobacterium marinum (WT) was grown in Middlebrook 7H9 broth (BD Biosciences, USA) supplemented with 0.5% glycerol and 0.05% TWEEN 80. 7H10 agar media supplemented with 0.5% glycerol and 10% OADC was used for growing mycobacterial culture on solid agar plates. The media was supplemented with hygromycin (50µg/ml) wherever required. *E. coli* strains were cultured in LB broth (Himedia). The media was supplemented with hygromycin (150 µg/ml).

2. Isolation of plasmid DNA

Steps:

- i. Pelleted down 10 ml bacterial culture overnight by centrifugation at 3500 rpm for 15 mins at 4°C, and the supernatant was discarded.
- ii. Resuspend pelleted bacterial cells in 300µl buffer P1 (Resuspension Buffer) and transfer to a microcentrifuge tube.
- iii. Add 300µl Buffer P2 (Lysis Buffer) and mix thoroughly by inverting the tube 4 to 6 times until the solution becomes clear. (Do not allow the lysis reaction to proceed more than 2 – 3 times)
- iv. Add 400µl Buffer N3 (Neutralization Buffer) and mix immediately and thoroughly by inverting the tube 4-6 times.
- v. And we centrifuged for 10 min at 13000 rpm ($\sim 17900 \times g$) in a tabletop microcentrifuge at RT.
- vi. Applied 800 µl of supernatant from Step 5 to the Qia prep 2.0 spin column by pipetting and centrifuging for 60 seconds and discarding the flow through, this was done for 2 times.
- vii. **Recommend:** Washed the Qiaprep 2.0 spin column by adding 500µl Buffer PB, centrifuged for 60s, and discarded the flow through, this was done 2 times.
- viii. **Reason:** This step is only required when using end A + strains or other bacterial strains with high nuclease activity (or carbohydrate content).
- ix. Washed the Qia prep column with diluted PE Buffer 1: 4 (100% ethanol) and centrifuged at 1300 rpm for 2 minutes at Room temperature, and centrifuged for 2 minutes to remove residual wash Buffer.

- x. Placed the Qia prep 2.0 column in a clean 1.5 ml microcentrifuge tube.
- xi. Finally, DNA was eluted by adding 40µl warm NFW to the centre of the Qia prep 2.0 column, after incubating the column for 5 minutes and then centrifuging for 1 minute at 13000 rpm, at RT.
- xii. Eluted DNA was stored at -20°C.

3. Preparation of Electro-competent cells of *Mycobacterium marinum* (Sahar H El-Etr *et. al.*, 2004)

- i. A 50 mL culture of *Mycobacterium marinum* at an optical density (OD₆₀₀) of approximately 0.4–0.8 was harvested.
- ii. Before preparing electrocompetent cells, both the 50 mL culture and the 10% glycerol solution were chilled on ice for 20 minutes.
- iii. The culture was centrifuged at 3,440 rpm for 20 minutes at 4°C using a swinging-bucket rotor.
- iv. The supernatant was carefully decanted, and the cell pellet was resuspended in 5 mL of ice-cold 10% glycerol. The final volume was adjusted to 40 mL with 10% glycerol.
- v. The cell suspension was washed twice with ice-cold 10% glycerol by centrifugation at 3,440 rpm for 20 minutes at 4°C.
- vi. Aliquots of 400 µL electrocompetent cells were prepared on ice and afterwards stored in -80°C.

4. Electroporation of plasmid DNA into competent cells (Sahar H El-Etr *et. al.*, 2004)

- i. For Electroporation, 400 ng of plasmid DNA were added to respective microcentrifuge tubes containing 400 µL of electrocompetent *M. marinum* cells.
- ii. Electroporation was performed using 2 mm gap cuvettes with the following parameters: 2.5 kV, 1000 Ω resistance, and 25 µF capacitance on Bio-Rad Gene Pulser Xcell.
- iii. The cells were then transferred to 1 mL of media and incubated for 5 hours at 30°C in a shaking incubator to revive.
- iv. Next, it was centrifuged at 3000 rpm for 5 minutes at RT.
- v. Pellet was formed, and the supernatant was discarded. Pellet was resuspended with the remaining media and then plated on antibiotic plates.

5. Growth Curve Analysis of *M.marinum* cells having pTEC17 and pTEC18 plasmids.

i. Initial Inoculation:

- Two colonies each from pTEC17-Neat and pTEC18-Neat plates were inoculated in 7H9 broth supplemented with Hygromycin B at a final concentration of 50 µg/mL.

ii. Subculturing:

- Subcultures were prepared when the optical density (OD600) of the parent culture reached approximately 0.5–1.0.
- The subcultures were inoculated at an initial OD600 of 0.05 using the parent culture.

iii. Growth Conditions:

- Each subculture was grown in 10 mL of 7H9 broth containing 10 µL of Hygromycin (50 µg/mL stock).
- Cultures were incubated at 30°C with shaking at 180 rpm.

iv. Time Point Measurement:

- The start of incubation was marked as 0 hours.
- OD600 measurements were taken at 5-hour intervals thereafter.

v. Sample Preparation for OD600 Measurement:

- At each time point, 500 µL of culture was mixed with 500 µL of sterile 7H9 broth to prepare a 1:1 dilution.
- A blank cuvette was prepared using 1 mL of plain 7H9 broth to calibrate the spectrophotometer (Eppendorf) at 600 nm.
- The 1 mL diluted sample was then transferred into a clean cuvette for OD600 measurement.

vi. OD Calculation:

- The observed OD600 value of the diluted sample was multiplied by 2 to obtain the actual OD600 of the original culture.

vii. Data Recording:

- Both raw (diluted) and corrected (actual) OD600 values were recorded at each time point.

6. Single Cell Suspension (SCS) of Bacterial Culture for Infection;

A. Subculture of Mycobacteria:

- On the day THP-1 cells are seeded, subculture *Mycobacterium marinum* (pTEC17 and pTEC18) into fresh 7H9 media at a 1:7 dilution ratio.
- Incubate at 30°C, shaking at 180 rpm for 4 days.

B. OD Measurement:

- After 4 days, measure the optical density (OD600) using a spectrophotometer.
- Use sterile 7H9 media as the blank.
- Acceptable OD600 range for infection: 0.5–1.0

C. Harvesting Bacteria:

- Centrifuge the cultures at 3500 rpm for 4 minutes at room temperature (RT).
- Discard the supernatant.

Note: This high-speed spin helps remove dead bacteria and pellet down viable cells.

D. PBST Washes (De-clumping and Cleaning):

- Wash 1:
 - Resuspend the bacterial pellet in PBST (0.05% Tween-80 in PBS) using the same volume as the original culture (e.g., 10 mL).
 - Mix thoroughly to break clumps.
- Centrifuge at 3500 rpm for 4 minutes at RT and discard the supernatant.
- Wash 2:
 - Resuspend the pellet again in 10 mL PBST and mix well.
 - Centrifuge at 3500 rpm for 4 minutes at RT and discard the supernatant.
- Wash 3:
 - Repeat the wash with 10 mL PBST.
 - Centrifuge at 3500 rpm for 4 minutes at RT and discard the supernatant.

Note: The second and third washes are critical for breaking bacterial clumps due to the action of Tween-80.

1 OD = 500 million bacteria

E. PBS Wash for SCS Preparation:

- Resuspend the pellet in 5 mL of tissue culture-grade PBS.
- Centrifuge at 800 rpm for 10 minutes at RT.
Note: The low-speed spin helps remove remaining clumps while retaining a single-cell bacterial suspension in the supernatant.
- Carefully collect the supernatant, which contains the Single Cell Suspension (SCS) of *M. marinum*.

Amikacin Treatment of Bacterial Culture (Pre-infection):

- To prevent extracellular growth and maintain MOI (Multiplicity of Infection), treat the SCS with Amikacin.
- Use:
 - **Stock concentration:** 10 mg/mL
 - **Working concentration:** 40 µg/mL
- Add an appropriate volume of Amikacin to the bacterial SCS and incubate before infection.

Infection of THP-1 Macrophages:**A. Infection Step:**

- Remove the existing media from THP-1 macrophage wells.
- Add the prepared infection media containing bacteria to each well.
- Incubate at 37°C for 3 hours to allow infection.

B. Post-Infection Amikacin Treatment:

- After 3 hours of incubation, remove the infection media from the wells.
- Add Amikacin-containing media at 40 µg/mL to each well to kill extracellular bacteria.
- Incubate at 37°C for 1 hour.

C. Final Wash:

- Remove the Amikacin media after 1 hour.
- Replace with fresh complete RPMI media for further incubation or analysis.

Result and Discussion:

- 1) THP-1 cells were seeded in 48-well tissue culture-treated plates following the standard protocol. The optical density (OD600) of the mycobacterial culture was measured at 0.558, and the corresponding single-cell suspension (SCS) had an OD600 of 0.156, equivalent to approximately 78 million bacteria per mL.

To achieve the desired multiplicity of infection (MOI), a total of 7.8 million bacteria were required per well. Accordingly, 100 μ L of the SCS was calculated and used per well. The SCS volume was mixed with complete RPMI media such that the final volume in each well supported proper infection while maintaining the intended MOI.

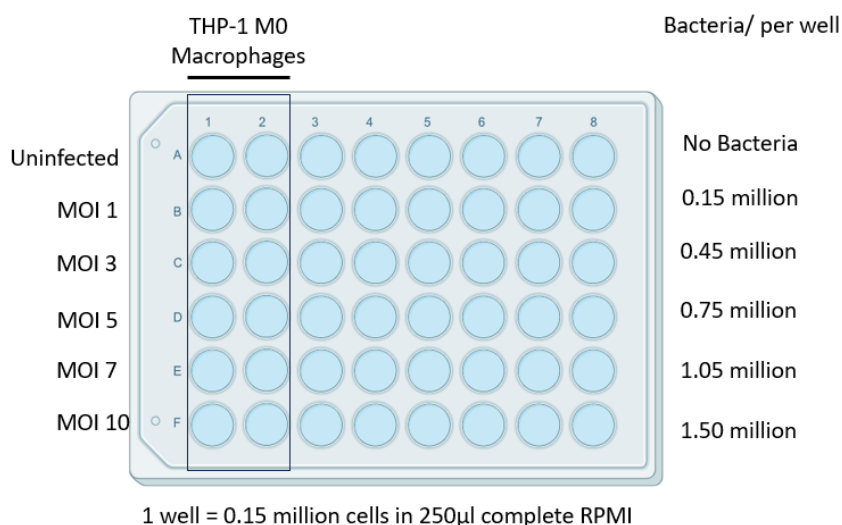


Fig: Plate layout showing THP-1 macrophages infected with *Mycobacterium marinum* at varying MOIs (1–10).

After completion of the Amikacin treatment, the THP-1 macrophages were replenished with fresh complete RPMI (cRPMI) supplemented with SYTOX Green, a fluorescent dye that selectively stains dead cells.

Fluorescence images were captured at 0 hours (immediately after Amikacin treatment was stopped) and again at 5 hours post-treatment. These time points were selected to assess the extent of infection and cell viability across different MOIs,

allowing visualization of the MOI at which the majority of cells remained infected yet viable.

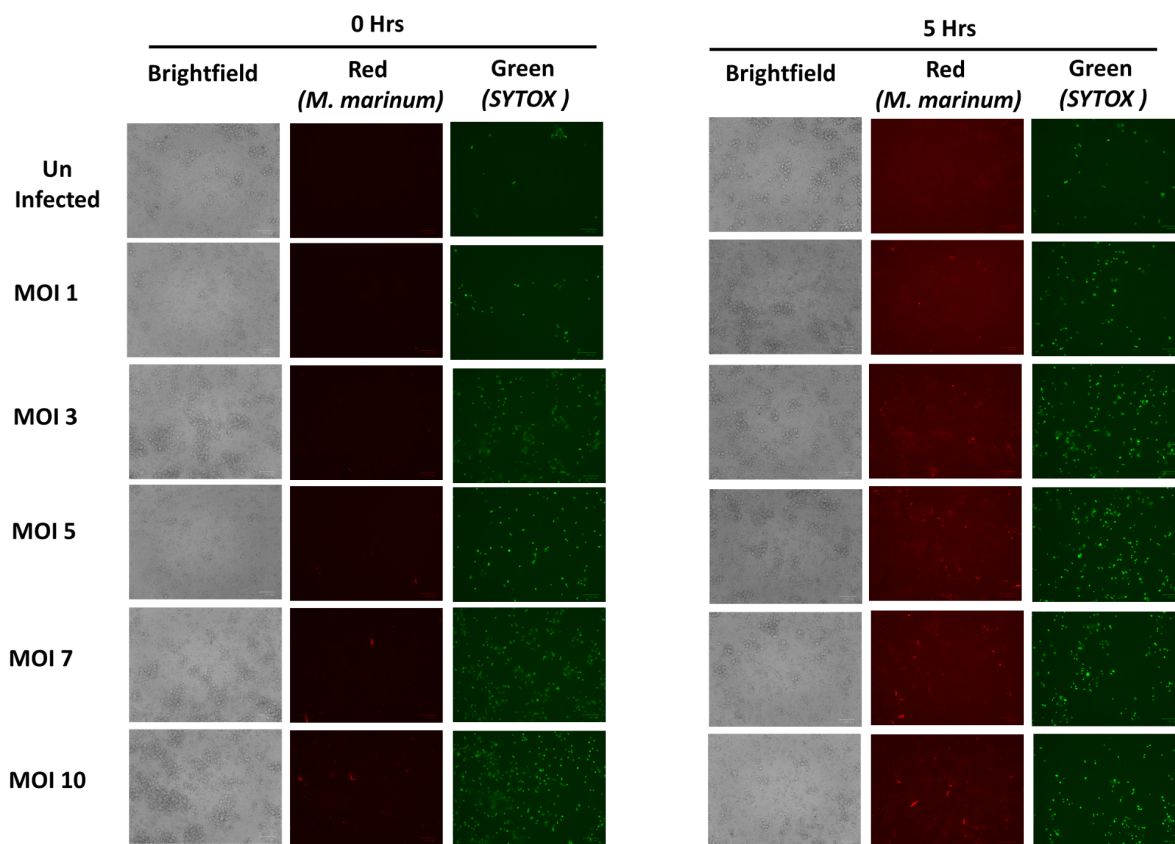


Fig: Zoe images of THP-1 Macrophages infected with *M. marinum* (red) at different MOI and stained with SYTOX Green (Green) at time 0 hrs and 5 hrs post Amikacin treatment.

mCherry Channel (Red Fluorescence): Red fluorescence intensity increases proportionally with MOI, confirming that higher MOIs correspond to a higher number of intracellular bacteria per cell.

SYTOX Green Channel (Green Fluorescence):

- At MOI 1–5, most cells remain SYTOX-negative (non-fluorescent), indicating that the majority of infected cells are viable and healthy at both 0 and 5 hours.
- At MOIs 7 and 10, there is a marked increase in SYTOX Green fluorescence by 5 hours, suggesting significant cell death at these higher MOIs.

- The increase in dead cells over time at high MOIs implies MOI-dependent cytotoxicity.

Phase Contrast Images: No major changes in overall cell morphology are observed between time points at lower MOIs, but higher MOIs (particularly MOI 10) show some signs of cellular disruption and reduced cell density by 5 hours.

2) Previously isolated pTEC17 and pTEC18 plasmids were quantified using Nanodrop, and size was determined by running a 0.7% agarose gel.

Nanodrop reading:

Plasmid	ng/ μ l	A260/280
pTEC17	125ng/ μ l	1.91
pTEC18	90 ng/ μ l	1.92

The extracted DNA was analysed on a gel, and 1 volume of orange G was added to 5 volumes of purified DNA. We mixed the solution by pipetting up and down before loading the gel.

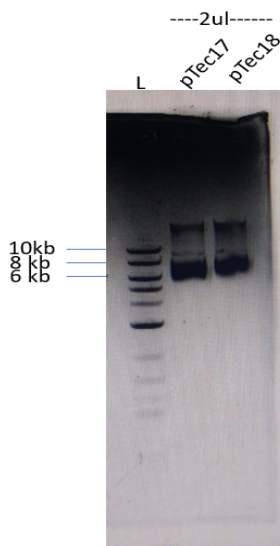


Figure 6: 0.7% Agarose gel picture of pTEC17 and pTEC18 plasmids

During Electroporation, the Time constant (ms) and Voltage values were as follows:

Sample	TC (ms)	Voltage
No DNA control	21.6	2482
pTEC17 (400ng)	21.0	2482
pTEC18 (400ng)	21.3	2483

A no-DNA control was also included. Post-electroporation recovery was carried out in Middlebrook 7H9 broth for 5 hours at 30°C with shaking. We plated neat and 1/10 dilution of pTEC17 and pTEC18 on 7H10 Hyg50 plates, also no DNA control on 7H10 and 7H10 Hyg50 plates. Then we keep these plates at 30° C. After 6 days, we get the transformation on the plates. We get the lawn on 7H10 plate (No DNA control) and no Colonies on 7H10 Hyg50 (No DNA control).

The following table shows the Number of colony counts:

Sample	Plate type	Colonies count
No DNA control	7H10	Lawn
No DNA control	7H10 Hyg50	00
pTEC17 (neat)	7H10 Hyg50	28
pTEC18 (neat)	7H10 Hyg50	32
pTEC17 (1/10 dilution)	7H10 Hyg50	08
pTEC18 (1/10 dilution)	7H10 Hyg50	02

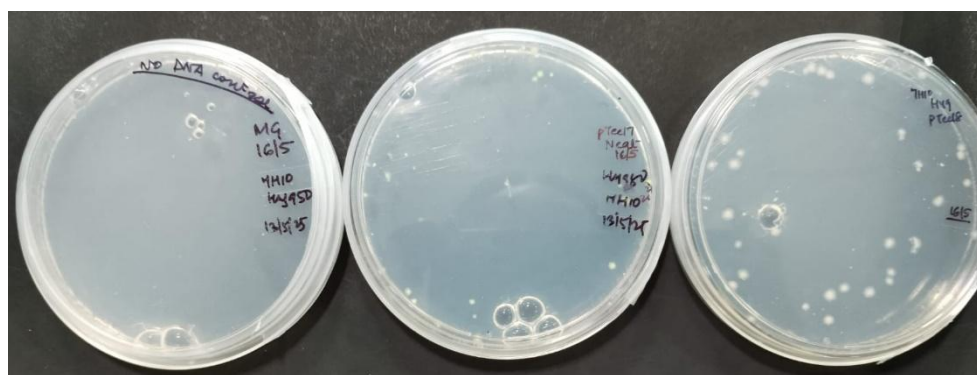


Fig 7: Images of 7H10 hyg plates having Transformants A) No DNA Control, B) *M. marinum* (pTEC17 -mTFP1), and C) *M. marinum* (pTEC18 -EBFP2)

pTEC17 harbouring *M. marinum* cells appear to be neon green in colour, whereas pTEC18 is white.

3) Growth curve of *Mycobacterium marinum*:

The growth curve of *M. marinum* includes four phases: **lag**, **log**, **stationary**, and **death**. In the **lag phase**, *M. marinum* adapts to the environment with minimal cell division. During the **log (exponential) phase**, it divides actively with a generation time of approximately **4-8 hours** under optimal conditions (25–35°C). In this phase, population doubling is consistent. In the **stationary phase**, nutrient limitation and waste accumulation slow growth, balancing cell division and death. Eventually, in the **death phase**, cell death exceeds division due to unfavorable conditions.

Time points and OD at 600nm were as follows:

Sl No	Time Point (hrs)	OD of pTEC17 <i>M.marinum</i>	OD of pTEC18 <i>M.marinum</i>
1	0	0.006	0.03
2	12	0.112	0.086
3	17	0.132	0.092
4	22	0.158	0.148
5	36	0.35	0.274
6	41	0.314	0.316
7	46	0.434	0.378
8	60	0.596	0.626
9	65	0.726	0.73
10	70	0.772	0.8
11	84	0.906	1.108
12	89	1.08	0.996
13	94	1.026	1.018
14	99	1.172	1.16

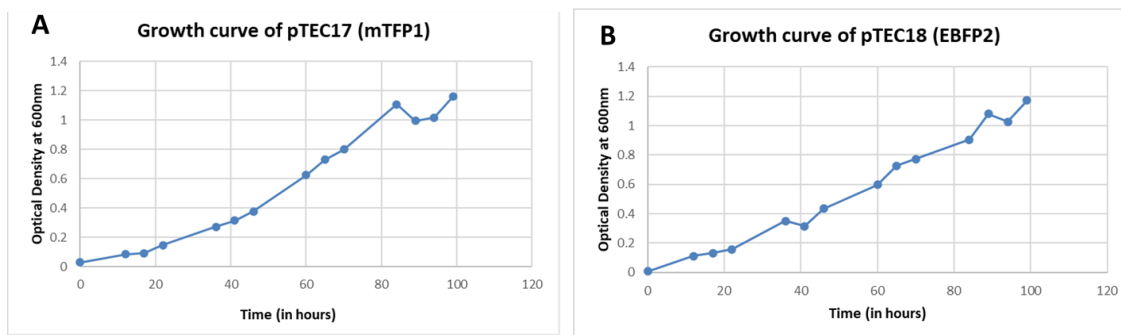


Fig 8: Growth curves of A) *M. marinum* (pTEC17 -mTFP1), and B) *M. marinum* (pTEC18 -EBFP2)

Doubling time is the time required for a bacterial population to double in number during the **exponential (log) phase** of growth. It is a key parameter that reflects how quickly a bacterial culture is growing under given conditions.

Doubling Time: $(\text{Time2} - \text{Time1}) / \log_2(B/A)$

OD at Time 2 - B, and Time 1 - A

Type of Plasmid	Time2-Time1	B/A	Log2(B/A)	Doubling Time
pTEC18	14	2.215189873	1.14743036365523	12.20117616
pTEC17	14	1.851351351	0.888578717331577	15.75549777

Doubling time was found to be 12 hours and 15 hours for *M. marinum* harbouring pTEC18 and pTEC17 plasmids, respectively.

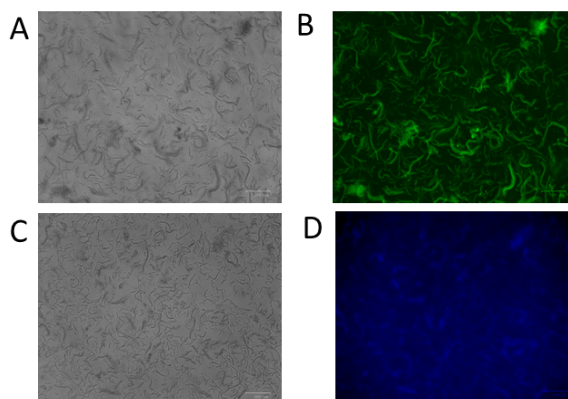


Figure: Zie-images of *M. marinum* expressing Teal fluorescent (A-B) and blue fluorescent protein (C-D)

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