STUDIES ON *MYCOBACTERIUM SMEGMATIS* BIOFILM AND ITS INHIBITION

Dissertation submitted in partial fulfillment of the requirement for the degree of

Master of Science

in

Biotechnology

By

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Under the supervision

of

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Declaration

I hereby declare that the work done by me on **"Studies on *Mycobacterium smegma*tis biofilm and its inhibition"** in partial fulfilment of the requirements for the award of the degree of **Master of Science in Biotechnology** submitted in the **Department of Biotechnology & Bioinformatics**, Jaypee University of Information Technology, Waknaghat is an authentic record of my own work carried out over a period from July 2024 to May 2025 under the supervision of **Prof. (Dr.) Rahul Shrivastava**, Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Solan, Himachal Pradesh.

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This is to certify that the above statement made by the candidate is true to the best of my knowledge.

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CERTIFICATE

This is to certify that the work reported in the M.Sc. Biotechnology thesis entitled **"Studies on *Mycobacterium smegmatis biofilm and its inhibition,*"** which is being submitted by **Mr. Avek Singh Halla (235111010)** in fulfilment for the award of Master of Science in Biotechnology by the Jaypee University of Information Technology, is the record of the candidate's work carried out by her under my supervision. This work is original and has not been submitted partially or fully anywhere else for any other degree or diploma.

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AVEK SINGH HALLA

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List of Abbreviations

NTM	**Non-tuberculous mycobacteria**
M. fortuitum	***Mycobacterium fortuitum***
M. smegmatis	***Mycobacterium smegmatis***
M. tuberculosis	***Mycobacterium Tuberculosis***
TB	**Tuberculosis**
E. coli	***Escherichia coli***
NA	**Nutrient Agar**
AIDS	**Acquired Immuno Deficiency Syndrome**
HIV	**Human Immunodeficiency Virus**
NAC	**N- Acetyl Cystine**
GLP	**Glycolipids**
MAC	***Mycobacterium avium* Complex**
M. surricatae	***Mycobacterium surricatae***
M. bovis	***Mycobacterium bovis***
M. microti	***Mycobacterium microti***
M. africanum	***Mycobacterium africanum***
M. avium	***Mycobacterium avium***
M. gordonae	***Mycobacterium gordonae***
M. xenopi	***Mycobacterium xenopi***
M. intracellulare	***Mycobacterium ntracellulare***
M. marinum	***Mycobacterium marinum***

M. orygis	***Mycobacterium orygis***
M. pinnipedii	***Mycobacterium pinnipedii***
M. mungi	***Mycobacterium mungi***
M. caprae	***Mycobacterium caprae***
HCL	**Hydrochloric Acid**
DNA	**Deoxyribonucleic Acid**
B. subtilis	***Bacillus Subtilis***
µl	**Microlitre**
Ml	**Mili litre**
RPM	**Revolutions Per Minute**
TAE	**Tris Acetate EDTA**
CFU	**Colony Forming Units**

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Abstract

M. smegmatis is considered a non-pathogenic bacterium. It is found everywhere in our surroundings. Also known as saprophytic mycobacteria, *M. smegmatis* is classified as a rapidly growing microbes that possess highly resistant cell membranes composed of mycolic acid lipids, making it highly adaptable in even the worst conditions. Also, these are aerobic, acid-fast bacteria that are highly resistant to acid decolorization and can only be visualized under special dyes such as carbon Fuchsin.

M. smegmatis is known as a model organism as currently it helps to determine the pathogenicity of various pathogenic species like *Mycobacterium tuberculosis,* etc. Due to its non-pathogenicity, it can be easily operated without any biosafety level. *M. smegmatis* resembles and shows more than 70% of identity with its cousin species. This study involves how the formation of biofilm occurs and methods to prevent or reduce the effects of biofilm formation and production.

Keywords: *M. smegmatis*, NTM, biofilms, *M. tuberculosis*.

CHAPTER 1
INTRODUCTION

1. INTRODUCTION

Nontuberculous mycobacteria (NTM) are usually species of bacteria that are non-pathogenic, that is, they do not cause any disease like *Mycobacterium tuberculosis* or *Mycobacterium leprae*. These are the types of mycobacteria usually found in nature, such as soil, water, pools, animals, etc. Mycobacteria that come under NTM are usually fast-growing mycobacteria, which mainly consist of species such as *M. smegmatis*, *Mycobacterium abscessus, Mycobacterium chelonae, and Mycobacterium fortuitum* [1].

At present, there are more than 140 NTM species present in nature. NTM are highly prevalent to pulmonary diseases in immunocompromised as well as immunocompetent patient, because NTM can survive in various bactericide used in water, such as chlorine, phenolic compounds therefore NTM keep themselves alive in water leads to cause various lungs diseases [1] Because they are significantly less pathogenic than *Mycobacterium tuberculosis*, patients with infections can be easily identifiable.

Because of their significantly lower pathogenicity than *Mycobacterium tuberculosis,* patients which are having infection can be easily identified due to the lower pathogenic nature of these species. They were observed in sixteen States, Australia, Russia, Canada, and Switzerland [2]. Long-chain (Carbon 60 to Carbon 90) of fatty acids make all mycobacterial species usually not hold out against to acid-alcohol decolorization; these types of bacteria are eventually visualized by the help of a special kind of dye, such as carbolfuchsin, under the microscope.[3]

While growing on provided media, *M. smegmatis* is usually observed in a white velvety and wrinkled structure [4]. After 2 days of growth of this mycobacteria appears abundantly and the colour that appeared to be white eventually changes to yellow in colour. NTM is mainly divided into two groups: rapidly growing mycobacteria and slow-growing mycobacteria. Mycobacteria such as *M. smegmatis* come under fast-growing mycobacteria [3].

M. smegmatis is known for studying the role of pathogenesis, the disease caused by *M. tuberculosis*. *M. smegmatis* is considered to be as a model microorganism to study the pathogenic species of Mycobacteria. To study the actual *M. tuberculosis* problem, arises that Tuberculosis can spread from one person to another. TB can cause two types of disease: pulmonary (in the lungs) or extra-pulmonary in other parts of the body other than the lungs)

[4]. However, to grow and study the TB is difficult as it is a slow grower and to grow on culture media it takes about 2-3 weeks, also having a generation time of 1 day.

Generation and combining the methods of tools of genetics and molecular biology for using different types of vectors. Due to its ability to be non-pathogenic, the use of biosafety level 3 is not necessary as used in pathogenic strains. *M. smegmatis* is considered to be a model organism because in the case of Mycobacteria, there are various conserved regions present in all the species of mycobacteria, for example in *M. smegmatis* and its related species of mycobacteria (pathogenic one also) most of them are having more then 70 -75 % of identity quotient, Also, it has more than 1200 of set of core proteins that resemble about of more than 50 % of amino acid identity [5]. Knowledge gap arises in *M. smegmatis* in case that it is not an optimum microbe while studying the pathogenesis. Because of its distantly related characteristics during case of growing period as well as in pathogenicity.

Aims and objectives:

At JUIT, Solan

- Studies on *M. smegmatis* biofilm formation and its inhibition
- To learn and perform basic microbiology techniques
- To learn and perform basic molecular biology techniques
- *M. smegmatis* biofilm formation and its inhibition studies

Internship at Corizo as a Business Development Associate

CHAPTER 2

REVIEW OF LITERATURE

2. Review of literature

2.1 Genus *Mycobacterium*

Initially, the mycobacterium species was named by Lehmann and Neumann in the year 1896 after the pathogenic strain that was *Mycobacterium tuberculosis.* The genus Mycobacterium is divided into two main groups on the basis of growth that is, rapid growers and slow growers. Most of the pathogenic species in Mycobacteria are slow growers as they take 2-3 weeks of time to grow on a culture medium [4]. On the basis of pathogenicity caused by the genus *Mycobacteria*, these are divided into three main types that are microbes which having harsh pathogenicity, also forming associations with the species of *Mycobacterium tuberculosis* complex and the species of *Mycobacteria* that cause a huge and adverse health effect on the worldwide population.

The *Mycobacterium* genus includes about 190 species, which include pathogenic as well as non-pathogenic species particularly *Mycobacterium* species is be included in the family of Mycobacteriaceae, followed by the order Corynebacteriales phylum/class Actinobacteria, and kingdom Bacteria. All mycobacterial species usually not hold out against to acid-alcohol decolorization; these types of bacteria are eventually visualized with the help of a special kind of dye such as carbolfuchsin, under the microscope [3].

2.2 *Mycobacterium tuberculosis*

Tuberculosis disease mainly occurs by the species included in the *M. tuberculosis* complex, which includes species such as *Mycobacterium africanum, Mycobacterium bovis, Mycobacterium canettii, Mycobacterium caprae, Mycobacterium microti, Mycobacterium mungi, Mycobacterium pinnipedii, Mycobacterium orygis,* and *Mycobacterium surricatae*[6]. *M. tuberculosis* is a slow-growing *mycobacterium* which is non-motile. Structurally, it is in rod shape and cells of the mycobacteria is made up of mycolic acid; therefore, also known as acid-fast bacterium. Nowadays, Tuberculosis is considered to be a highly infectious disease, surpassing HIV and AIDS rapidly, across the world population. TB is reviewed as the top 5 diseases involved in causing death.

According to the reports by 2018, around 10 million of the population across the globe got infected with TB, out of which 1.2 million people died.

Two-thirds of the cases was reported to be appeared in eight highly populated countries, India (27%) and China (9%) [7] whom are ranking at the top of the list. *M. tuberculosis* mainly causes two types of infections that is pulmonary (lung infection) and extra-pulmonary (in other parts of the body other than the lungs). TB occurs most often in immunocompromised individuals but can also infect immunocompetent individuals. The latest report on TB in 2024 depicted that in 2024 around the world 193 countries and areas which is more than 99% of the total population is reported TB cases [7].

The infectious rate of TB is adverse in that it usually affects 10 per 100,000 in North America to 10 to 300 per 100,000 in Asia and Western Russia to over 300 per 100,000 in Southern and Central Africa. Around the worldwide population, the stat is reported that in every 15 seconds, one person loses their life. If TB is detected initially it can be curable within 6 months of the diagnosis; however, the patients who do not undergo any treatment 60% of them die because of the disease.

In diseases like TB pathogens largely affect the lungs where the alveoli present inside engulf the macrophages of mycobacteria. As the bacteria enter the host, they start to divide themselves in large numbers which makes them resistant easily to the innate immunity of the individuals [8].

Initially, the molecular method used to diagnose TB was Mantoux's test. It diagnoses the disease by determining the amount of T-cell response induced against the pathogen.

Mycobacterium tuberculosis can be treated if diagnosed early. Treatment of TB can be done only by eliminating the active form of TB present in the host body. Nowadays the treatment that is performed is usually on the concept of monotherapy with Isoniazid due to the pathogenicity of the pathogen in the host body this type of treatment can distinctly vary from the period of 6 to 9 months. Related treatment to eliminate the TB infection is by having the course of Rifampicin for 4 months alone or having Rifampicin in combination with pyrazinamide for about 2 months [9].

2.3 NON-TUBERCULOUS MYCOBACTERIA

Nontuberculous mycobacteria (NTM) are mostly non-pathogenic *Mycobacteria*. NTM are considered to be *Mycobacteria* that only get metabolically activated if they find any chance to grow in the environment. Also, NTM is found in the environment everywhere like water, soil, ponds, and sometimes in animals, as well. NTM are associated with the genus *Mycobacterium. Mycobacteria* are observed as non-motile, aerobic, and have thick cell walls consisting of mycolic acid. The most widely found species in NTM are *Mycobacterium intracellulare*, *M. kansasii*, *M. fortuitum*, *Mycobacterium abscessus*, and *Mycobcterium chelonae*.

NTM comes under the category of fast growers, which means they grow on a culture medium within 7 days, most of the time NTM does not show pathogenicity, so working with NTM is easier and also does not require biosafety level 3. In the last decade studies were conducted to determine the prevalence of NTM over tuberculosis as per the 22 studies which were conducted from 1946 to 2014 there was a huge decrease in no. of TB was observed that was about 81% whereas on the other hand, NTM rises around the world about 92% in all the geographical areas [10].

NTM can affect both immunocompromised as well as immunocompetent individuals but occurs more often in immunocompromised hosts, especially with some pre-existing disease such as HIV or diabetes mellitus. The molecular diagnosis of NTM is somewhat different from actual TB disease and comparatively difficult in the case of NTM because it seems difficult to distinguish between the mutualistic microbes present inside the host and the actual pathogenic microbes it does happen due to the host's interaction with the environment [10],[11].

NTM has become a highly pathogenic disease in recent times therefore prevention of the disease is becoming more difficult due to the late diagnosis as well the *Mycobacteria* are adapting well to the environment therefore becoming adaptable to the environment and showing antibiotic resistance to various broad-spectrum antibiotics. Another reason that makes the treatment of NTM difficult is that if the patient has any active pathogenic strain left in the body after injection with antimicrobial treatments, it can show more fatal signs in the host’s body, which can be deadly and harmful [6].

2.4 EPIDEMIOLOGY OF NTM

In the last few decades, the increase in prevalence of NTM has remained unnoticed which leads the NTM to surpass the TB cases worldwide. Most of the cases in the world arise in immunocompromised patients who have some pre-existing disease like HIV or AIDS. Some of the studies have determined that in the previous 3 to 4 decades, cases of NTM have increased throughout the globe very rapidly. Cases of NTM have increased exponentially in North America from 9.1 to 14.1 per 100,000 persons/year between 1997 and 2003 as well in the last decade of the NTM in USA is reported as 1.4 and 6.6/100,000 individuals, whereas in countries like UK there was about 4/100,000 to 6.1/100,000 individuals between 2007 and 2012[10]

In the case of NTM organisms usually form an association. according to the reports, it is considered that NTM forms an *M. avium* complex (MAC), which includes half of the NTM strains collected from host bodies globally. 31% of strains were collected from South America, 52% from North America, and 71% from Australia, as NTM is not known most often for pulmonary infection, but many of times NTM can also initiate pulmonary infection in the absence of any incurable disease. NTM prevalence also varies from person to person based on age and sex it is observed that in women, there are 59% chances of NTM disease across the globe, which is also the same in elderly persons [9].

According to the reports, there are different species included in MAC, which are usually collected from the patients in which widely found species observed in patients' body is *M. chelonae* and *M. abscessus* (12.1%), followed by *M. fortuitum* (5.6%), along with *M. kansasii* (5.5%).

2.5 *Mycobacterium smegmatis*

M. smegmatis is a non-pathogenic species associated with NTM. It is considered an opportunistic mycobacterium, and it is usually found in the surroundings, everywhere soil, water, etc. It is considered to be as the model organism for study because it does not cause any disease and also has less generation time which makes *M. smegmatis* grow easily within 7 days. The Mycobacteria genus consists of conserved regions in every species; therefore, various characteristics are similar in the majority of species, *M. smegmatis* is an aerobic bacterium with a rigid cell wall consisting of mycolic acids. Lehmann and Neumann coined the name *M. smegmatis* in 1889[3].

Initially, to study various aspects of pathogenic strains of mycobacteria species of *M. smegmatis* was genetically modified to mc^2 155. Crawford and Bates originally found this species of *M. smegmatis* from the mycobacteria's plasmid.[11] In a modified strain of *M. smegmatis* (mc^2 155), the cell wall of the *mycobacteria* was also studied to determine the passage of antibiotics through the membrane of mycobacteria by creating pore protein to reduce the obstruction of antibiotics inside the cell membrane [12]. Also, it is a perfect model because operating this species is easy and does not require biosafety level 3, also it can grow at temperatures between 55 degrees Celsius to 60 degrees Celsius.

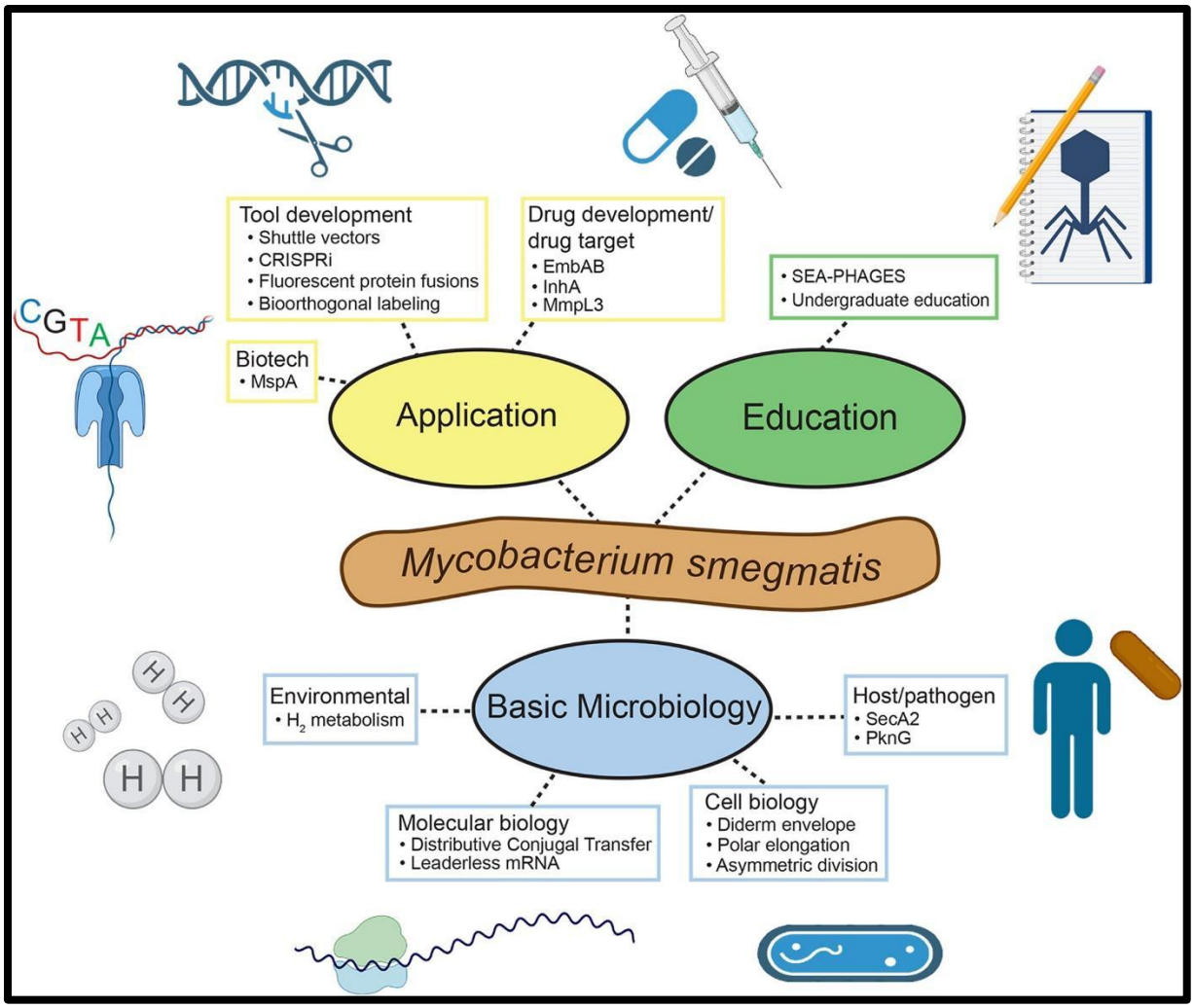


Figure 1: Illustration of *M. smegmatis* as a role model organism.[5]

M. smegmatis considered way better model organism then other organisms because it differentiates between the gene products, it determines about the interaction that occurs between the pathogen and the body of the host which usually gets infected, also *M. smegmatis* is designated as a model organism because it also works on toxicology broadcast and further use of that centred approach for the drug which shall be safe and effective [5].

Studying *M. tuberculosis* or any pathogenic strain is easy via *M. smegmatis* because, in the case of the Mycobacteria genus, there are highly conserved regions present that resemble about, more than 60 % also the regions which are required for the study in *M. smegmatis* 95% and same region resembles in *M. tuberculosis* which comprises of about 90% of them. However, *M. smegmatis* shows more than 50% indistinguishability in the case of amino acids when compared to species like *M. abscessus*, *Mycobacterium marinum*, *M. avium*, and *M. leprae*.

2.5.1 Cell wall research on *M. smegmatis*

The Mycobacteria genus comprises of thick cell wall consisting of mycolic acids, which makes the structure of the cell wall of mycobacteria highly hydrophobic in nature also making cell wall resistant to any kind of foreign substances as well as allows to sustain in harsh environments. It gets distinguished with the cell walls of normal bacteria in case the cell wall of mycobacteria consists of a greasy cell wall which is composed of lipids which usually present in the conserved region of *mycobacteria* [13].

To reduce the resistance of the cell wall of mycobacteria idea of a pore-forming protein was constructed that resembles to the cell wall of gram-negative bacteria. MC^2155 was used and after conducting the study on the cell wall proteome of *M. smegmatis* can be beneficial by giving insight against infection-causing *Mycobacteria* and how to tackle the disease-causing species. All mycobacterial species are usually hold out against to acid-alcohol decolorization; these types of bacteria are eventually visualized with the help of a special kind of dye, such as carbolfuchsin under the microscope.

2.5.2 Infection caused by *M. smegmatis*

M. smegmatis is a non-infectious mycobacterium but sometimes it can cause infection it was collected from the genitals. Gordon and Smith in 1953 were able to research more briefly on the different aspects of *M. smegmatis* also *M. smegmatis* can cause pleuropulmonary and soft skin tissue which was reported in 1986[14].

As we know, *M. smegmatis* is present everywhere in the surroundings; therefore, the prevalence of the skin infection is increasing in number as infection can easily spread on inanimate things used in skin treatment, more rapidly in susceptible individuals. It was believed that *M. smegmatis* is non-infectious, but due to the lack of knowledge about this mycobacterium is gaining a number in cases due to unsterilized skin treatment operations. However, in countries like China, the USA as well as in European countries few cases related to skin have been observed [15].

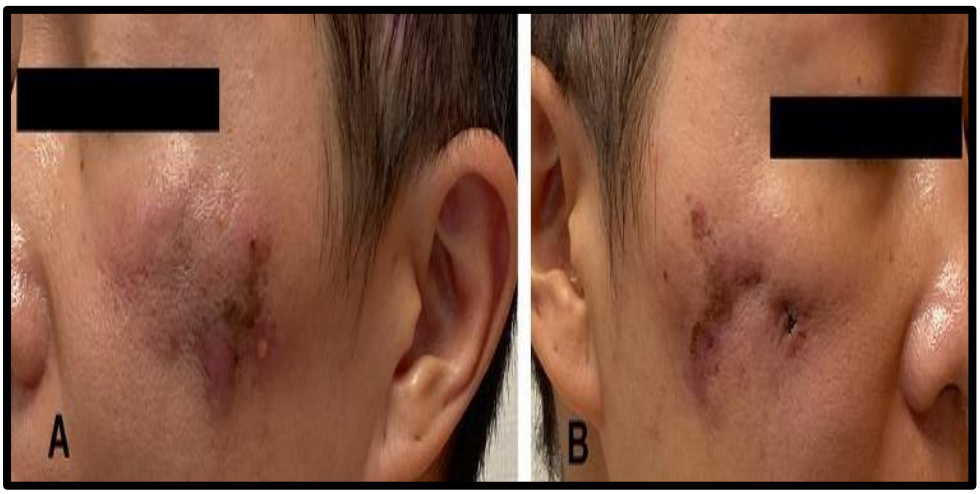


Figure 2: Skin infection due to M. smegmatis via skin injections [15]

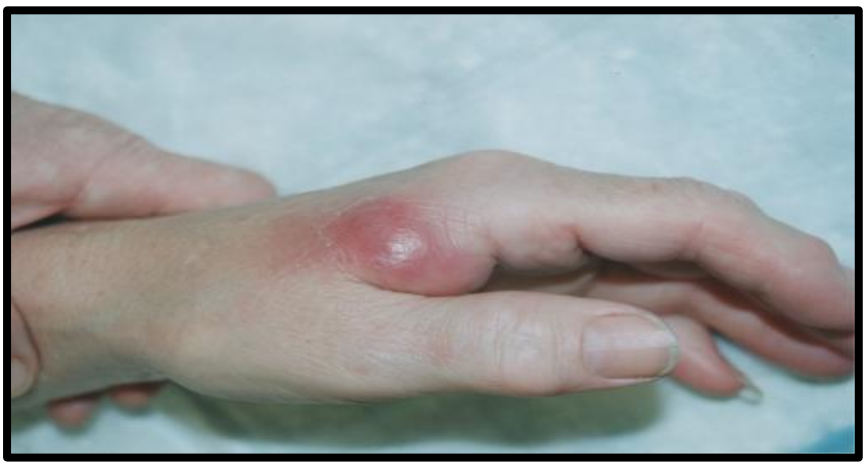


Figure 3: Skin infection on soft tissue [16]

2.5.3 Biofilm in mycobacteria and *M. smegmatis*

Biofilms are said to be the social organization of microbes which makes them survive even in extreme environments and also makes them resistant to a variety of antibiotics, initially, bacterial colonies are present in free-floating form and afterward get attached to the surface resulting in the formation of a social organization known as biofilms. The first case of biofilm formation was observed in 1978[17]. Mycobacterial biofilm is however, different from normal bacterial biofilm as mycobacterial biofilm is highly resistant due to the composition of lipids present in their cell wall. Also, microbial aggregates form a matrix by the release of exopolysaccharides, there is the transport of ions, food, etc. between the microbial colonies inside the biofilm.

Mycobacteria have an ability that is they can grow and form biofilms on inanimate things such as surgical devices, etc. The main diseases that occur by biofilm are usually pulmonary diseases cystic fibrosis is one of them, Biofilm mainly affects immunocompromised more often or hosts having infectious conditions, but can also affect immunocompetent also [16]. For being antibiotic-resistant microbes perform various mechanisms such as efflux pumps, porin loss, and quorum sensing are a few of them. In this mechanism, bacteria detect antibiotics as foreign material and try to eliminate them as quickly as possible. Both Infectious as well as NTM can efficiently form biofilms. some of the bacteria that are highly prevalent in biofilm formation and have become highly resistant to various broad-spectrum antibiotics are *M. tuberculosis, M. leprae, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecium, etc.*

However, molecular diagnosis depends upon the patient's condition the person infected with biofilm with develop certain symptoms such as cough, hemoptysis, weight loss, fever [17]. Diagnosis in clinical is done by observing chest X-rays and by culturing methods. In such cases of biofilms, antibiotic treatment does not show effectiveness as microbes have created ways to resistance to various broad-spectrum antibiotics, also microbial colonies in attached form require a heavy dose of antibiotics for a longer time. To tackle antibiotic biofilms new techniques were established such as the use of molecules that can disintegrate the biofilm formation such as N-acetylcysteine (NAC) and Tween 80, which can be given in combination with antibiotics other techniques to act as antibiofilm are nanoparticles, phototherapy, phage therapy, vaccines, antimicrobial peptides, and new antibiotics [18].

It is proven that not only pathogenic species but also rapidly growing mycobacteria (NTM) can also contribute form biofilms, such as *M. smegmatis*, *M. fortuitum, M. chelonae*, and *M. marinum,* because these *mycobacteria* are everywhere in the surroundings, as well as mycobacterial biofilms are hugely facilitated by the glycolipids (GLP).

Biofilm formation occurs in a defined order: sessile formation, bacterial adhesion, microcolony formation, maturation, and dispersion. It is nearly impossible to treat biofilm after it transforms from free-floating to sessile, becoming irreversible. While the creation of biofilm during the adhesion step *M. smegmatis* GLP is considered to be an initial requirement. *Mycobacteria* keep multiplying and reproducing in biofilms. there was a report that reaching the maximum growth of *M. smegmatis* takes about 90 to 95 hours.

In recent studies, to reduce the formation and biofilm and its effect of biofilm formation was performed on the autolysis of the functional part of mycobacteria. It was determined that autolysis of a specific gene in *M. smegmatis* is MSMEG_6935. Deletion of the autolysis gene results in restriction of pathogenic protein, mainly the CwlM protein is encoded by *M. smegmatis*. In recent times, various ways to tackle biofilms have been established. The Initial step of the disruption of biofilm is to hack the machinery of bacteria reproducing in the biofilm matrix. For the restriction of *M. smegmatis,* the by-product of Quinazole is used in a non-pathogenic way. It is seen that Ophiobolin K weakens the biofilm structure and also retains the antibiotic effect in *M. smegmatis*.

CHAPTER 3

Materials and methods

3. Materials

3.1 Microbial Strain

Table 1: Microbial strains

Strain	**Sources**
E. coli DH5α	IMTECH (Institute of Microbial Technology), Chandigarh, India
Bacillus subtilis	IMTECH (Institute of Microbial Technology), Chandigarh, India
Mycobacterium smegmatis MC^2155	Central Drug Research Institute (CDRI), Lucknow, India

3.1.2 Media

Table 2: Media

Nutrient Agar	HIMEDIA
Nutrient Broth	HIMEDIA
Luria Broth	HIMEDIA

3.1.3 Chemicals

Table 3: Chemicals

Crystal Violet	Loba Chemie
Gram's Iodine	HIMEDIA
Safranin	HIMEDIA
Basic fuchsin	HIMEDIA
Methylene Blue	Fisher Scientific
Tween 80	SRL
Agarose	HIMEDIA

Sodium Chloride	Merck
SDS	Bio Red
Saturated Phenol	SRL
Chloroform	SRL
Isoamyl	SRL

3.2 Methods

3.2.1 Techniques in microbiology

3.2.1.1 Media Preparation

3.2.1.2 Nutrient agar

1. A clean beaker was taken, and 13 grams of Nutrient Broth powder was added to 1 L of distilled water.
2. Also added 20g of agar to 1 L of solution.
3. The dissolved medium was then autoclaved at 15 lbs pressure (121°C) for 15 minutes.
4. Once the autoclaving process was completed, the beaker was taken out and cooled to room temperature.
5. Autoclaved media was stored at 4°C.

3.2.1.3 Nutrient broth

1. Added 13 grams of nutrient broth in 1 L of distilled water in a cleaned flask.
2. The media was mixed and dissolved completely.
3. The flask was covered with a cotton plug.
4. Sterilized by autoclaving at 121°C for 15 minutes.
5. Once the autoclaving process was completed, the flask was taken out and cooled to room temperature.
6. Autoclaved media were stored at 4°C.

3.2.1.4 Luria broth

1. Added 25 grams of Luria broth to 1 L of distilled water in a cleaned flask.
2. The media was mixed and dissolved completely.
3. The flask was covered with a cotton plug.
4. Sterilized by autoclaving at 121°C for 15 minutes.
5. Once the autoclaving process was completed, the flask was taken out and cooled to room temperature.
6. Autoclaved media were stored at 4°C.

3.2.1.5 Media preparation for *M. smegmatis*

1. A clean beaker and 13 grams of Nutrient Broth powder were added to 1 L of distilled water.
2. Also added 20g of agar to 1 L of solution.
3. 0.5 ml of Tween 80 was added to the medium in the flask containing 1 L of solution.
4. Sterilized by autoclaving at 121°C for 15 minutes.
5. Once the autoclaving process was completed, the flask was taken out and cooled to room temperature.
6. Autoclaved media was stored at 4°C.

3.2.2 Autoclave sterilization

1. First, everything that needed to be sterilized inside the chamber and the lid of the autoclave.
2. A vacuum pump sucks the air out of the chamber. The steam entering the chamber – and the pressure it creates – also work to remove the air.
3. The autoclave sterilization was performed at 15 psi for 15 minutes.
4. After 15 minutes, wait for another 20 minutes.
5. The steam was released with the help of a steam valve and the chamber could be opened. The freshly sterilized materials can be removed.

3.2.3 Simple streaking

1. Nutrient agar media was poured onto a sterilized Petri plate and labelled properly
2. The inoculation loop was sterilized till red hot on the flame of a Bunsen burner.
3. After cooling down the loop, an isolated colony of bacteria was picked by a loop from the cultured plate.
4. The loop was dragged gently on the nutrient agar plate in a zig-zag pattern.
5. The loop was again sterilized until it became red hot.
6. At last, Petri plates were kept in an incubator for overnight at 37°C.

3.2.3.1 Quadrant Streaking

1. Nutrient agar media was poured onto a sterilized Petri plate and labelled properly
2. The inoculation loop was sterilized till red hot on the flame of a Bunsen burner.
3. After cooling down of the loop, an isolated colony of bacteria was picked by a loop from the cultured plate.
4. Smear was prepared at one end of the Petri plate and dragged to the other end.
5. The loop was again sterilized until it became red hot.
6. The plate was rotated 90 degrees, and other streaks were made from the end of the previous streak.
7. The Petri plate was labelled as four different quadrants, and the technique of dragging from one end to the other, where the last streak was left.
8. A similar procedure was repeated 3 times.
9. In the last step Petri plate was incubated overnight at 37°C.

3.2.4 Gram staining

1. A clean glass slide was prepared.
2. One drop of autoclaved distilled water was kept on the slide and a thin smear was prepared by taking culture from the tube with an inoculation loop.
3. The slide was kept for air drying and fixed with heat properly over the Bunsen burner.
4. Crystal violet dye was poured on the slide, and the slide was kept for about 45 seconds to 1 minute, and then washed with tap water gently.

5. Gram's iodine mordant dye was poured on the slide and kept for 1 minute, and again rinsed with tap water.
6. Then the slide was washed with decolorizer, which was 95% alcohol, for about 35 seconds, and the slide was once more rinsed with water.
7. Counterstain safranin dye was poured and kept for about 40 seconds, and washed with tap water.
8. The slide was kept for air drying properly.
9. After that slide was observed under the light microscope.

3.2.4.1 Acid-fast staining

1. A clean glass slide was prepared.
2. One drop of autoclaved distilled water was kept on the slide and a thin smear was prepared by taking culture from the tube by an inoculation loop.
3. The slide was fixed with heat properly inside the laminar airflow.
4. After the slide was dried, the slide was placed on wire gauze and a hot plate.
5. Flooded the smear with carbol-fuchsin.
6. The slide was rinsed with running tap water gently.
7. The slide was washed with decolorizer (3% conc HCl in 96% Ethanol) and then rinsed with water.
8. The cell was counterstained with Methylene Blue for about 2 minutes.
9. The slide was washed with tap water again.
10. The slide was kept for air drying.
11. After that slide was observed under the light microscope.

3.2.5 CFU Count from Soil Sample

1. The sample was taken in a sterilized test tube from campus, from the soil.
2. In test tubes, 9 mL of autoclaved water was taken in each tube.
3. A 1ml sample was taken from the first test tube and transferred to the second tube, and then all the tubes were serially diluted simultaneously, for example: 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8},10^{-9},10^{-10}
4. From each tube including neat 200µl of sample was spread on different plates containing nutrient agar.
5. The sample was uniformly spread on the plates with the help of an L-shaped spreader.

6. Plates were kept in an incubator at 37℃ for 24 hours.
7. After 24 hours, the colony was counted from plates, and CFU was calculated.

Molecular biology techniques

3.2.6 Genomic DNA isolation from *E. coli*

1. The *E. coli* sample was inoculated in Luria broth and kept for incubation for 24 hours at 37℃.
2. After 24 hours, the *E. coli* culture was transferred to the 2 mL Eppendorf tubes and centrifuged for five minutes at 12000 rpm at room temperature.
3. The Supernatant was discarded, and the pellet was kept.
4. 500µl of extraction buffer and 50 µl were added to the pellet and mixed by vortexing.
5. After mixing, the tubes were incubated at 65℃ for thirty minutes in a water bath.
6. Tubes were kept in a water bath till the sample was cleared.
7. After incubation, 550µl of Phenol and Chloroform (1:1) was added into the tubes.
8. Tubes were centrifuged at 10,000 rpm for five minutes at Room temperature. Two layers were formed.
9. The upper layer (Aqueous layer) was transferred to the fresh Eppendorf tubes.
10. Took 400 µl of sample from an aqueous layer in another Eppendorf tube of 1.5 ml.
11. To the tubes intended for the precipitation of DNA, a double volume of cold isopropanol was added with 40 µl of 5M NaCl.
12. Tubes were kept at -20℃ for 20 hours.
13. Tubes were again centrifuged at 12000 rpm for fifteen minutes at 4℃.
14. The supernatant was discarded, and the DNA pellet was rinsed with 1 mL of 70% ethanol.
15. Eppendorf tubes were centrifuged again at 12000 rpm for five minutes at 4℃.
16. DNA pellets are kept for air drying by inverting the tubes.
17. The pellet was dissolved in nuclease-free water.
18. After that tubes were stored in a 4℃ refrigerator.
19. The 0.8% Agarose was prepared for gel electrophoresis
20. 1µl of Ethidium bromide was added to the agarose.
21. The gel was kept in an electrophoresis chamber, and 1X TAE was added.

CHAPTER 4

RESULTS

4 Results

4.1.1 Simple Streaking

Cultures of *E. coli, B. subtilis* were streaked on NA (Nutrient agar). Images of culture plates [Fig. 4] are shown below.

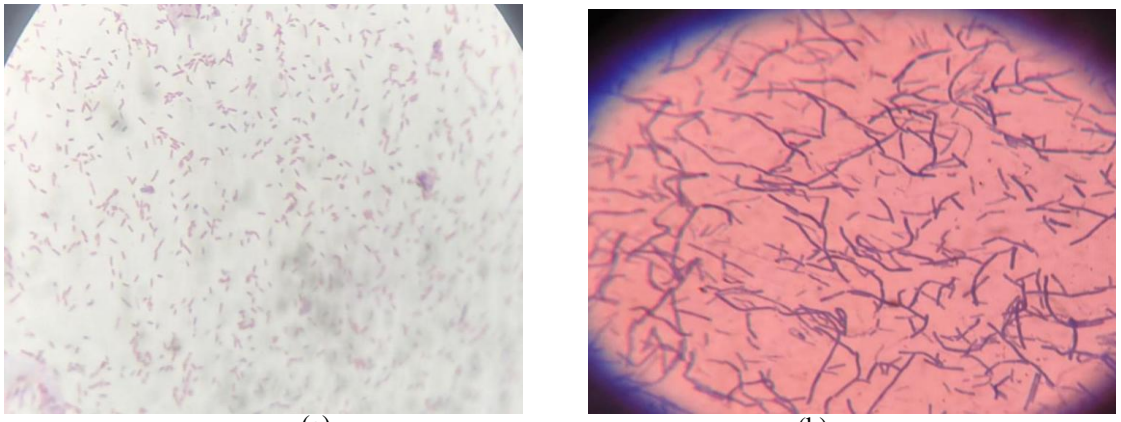
Figure 4: Simple streaking of *E. coli*

4.1.2Quadrant streaking

(a) (b)

Figure 5: (a) Quadrant Streaking of *E. coli* and (b) Quadrant streaking of *M. smegmatis*

4.2 Gram Staining



(a) (b)

Figure 6: The figure shows that (a) Gram staining of *Bacillus subtilis* and (b) Gram staining of *E. coli*.

4.2.1 Acid-fast staining

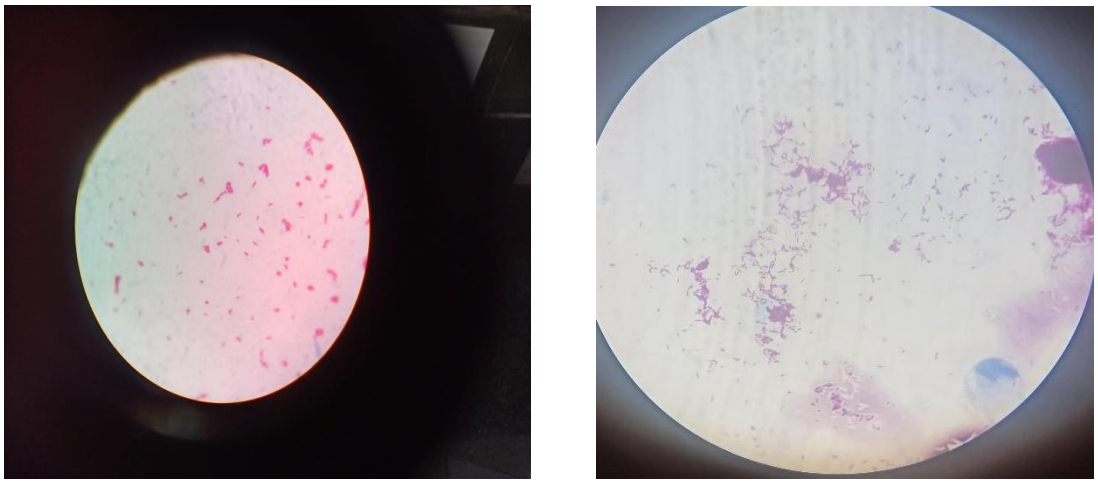


Figure 7: Ziehl-Neelsen staining of *M. smegmatis*

4.3 CFU count of soil sample

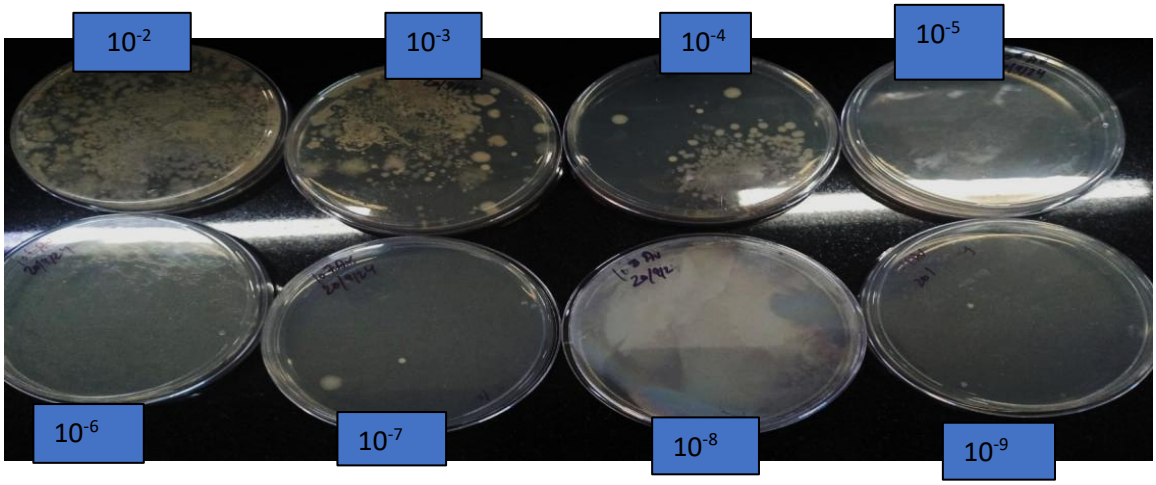


Figure 8: Petri plates showing colonies obtained on spreading of different dilutions of the soil sample.

4.4 CFU count of *E. coli*

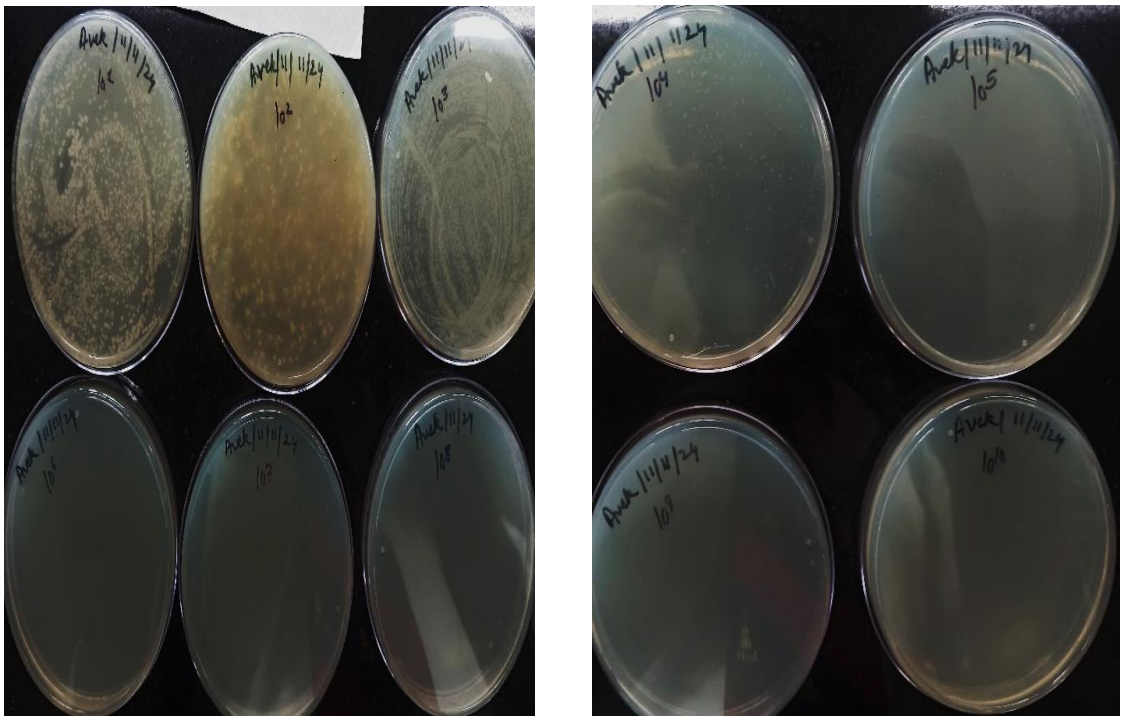


Figure 9: Petri plates showing colonies obtained on spreading of different dilutions of the *E. coli* sample.

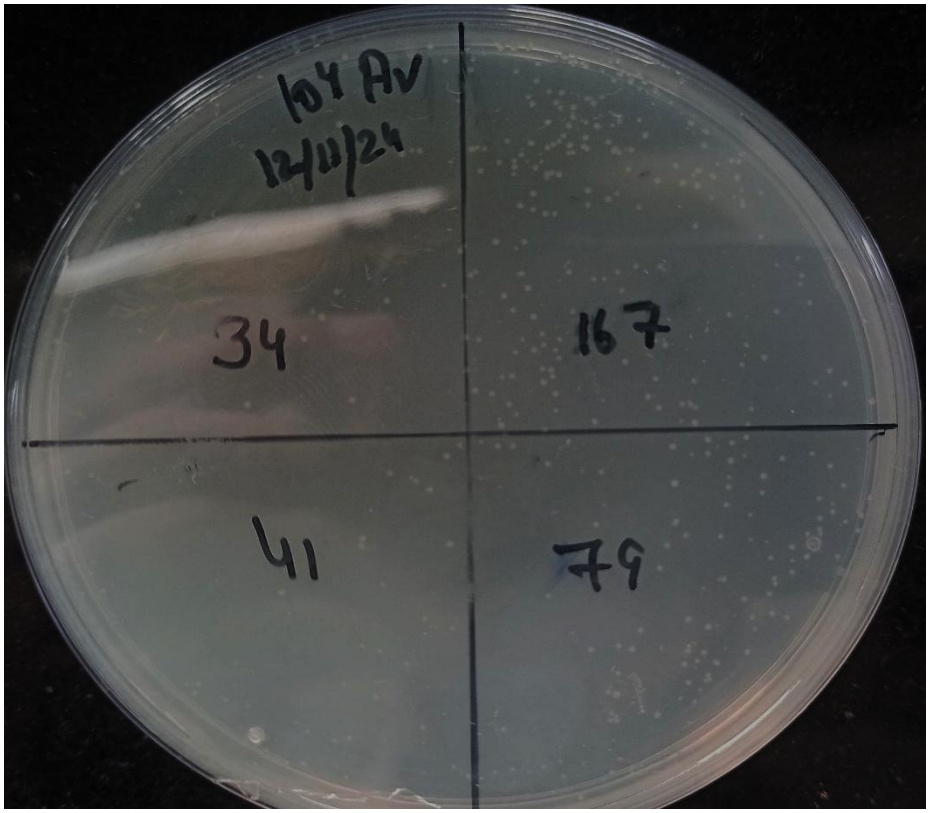


Figure 10: Image showing countable colonies in 10^{-4}

$$\text{CFU/mL} = \frac{291 \times 10^{-4}}{0.1}$$

$$= 2.9 \times 10^{-5} \text{ CFU/ml}$$

Table 4: Colony Forming Units Count at Different Serial Dilutions

Dilutions	No. of colonies
10-1	Not countable
10-2	Not countable
10^{-3}	Not countable
10^{-4}	291
10^{-5}	25
10^{-6}	0
10^{-7}	0
10^{-8}	0
10^{-9}	0
10^{-10}	0
10^{-9}	0
10^{-10}	0

4.5 Isolation of genomic DNA from *E. coli*

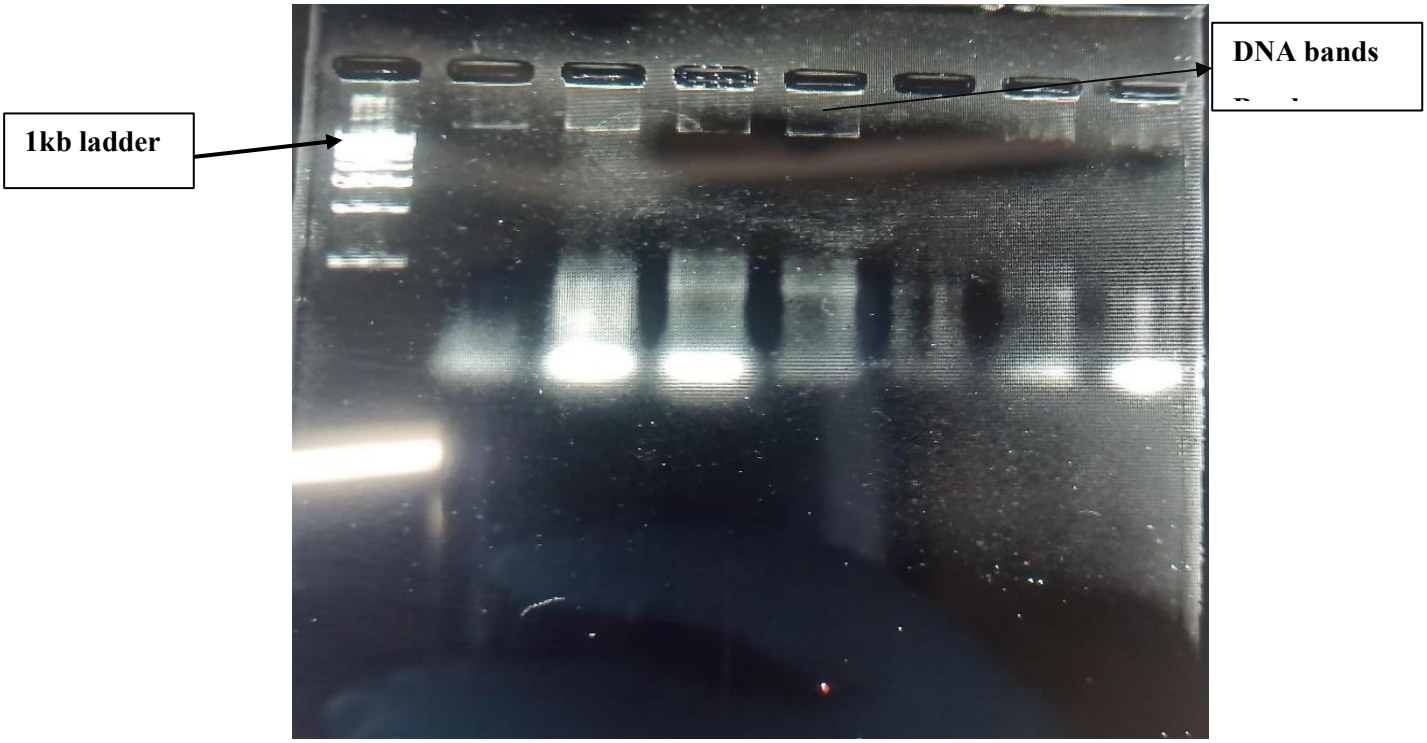


Figure 11: Illustration of genomic DNA isolation

CHAPTER 5

Introduction of Company and Industry

5.1SelectionProof

CORIZO

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Bengaluru, Karnataka 560068

19 December, 2024

PROBATION OFFER LETTER

Dear **AVEK SINGH HALLA,**

Congratulations! We are pleased to offer you to join **Corizo Edutech**. It was great to interact and exchange thoughts on how to make this relationship win: win. Please go through the below details and let us know if you have any queries and/or acknowledge your agreement to the same.

Please find the details of your internship below:

1. **Date Of Joining:** 15-1-2025
2. **Designation:** Business Development Associate
3. **Location:** Gurugram
4. **Compensation Offered:** Fixed CTC - INR 400,000 LPA plus a Variable CTC of INR 250,000 LPA which is uncapped as of date.

Probation :

- You will be under probation for a period of 90 days (3 months) from the date of joining and will be confirmed after a review of your performance during the probationary period.
- During the period of probation your monthly CTC will be INR 15,000 + incentives upto INR 10,000.

Period of Service:

- The minimum period of service is three (3) months from the date of enforcement of this offer letter.
- The minimum period of service is not negotiable.
- The first ten days will be on job training (OJT) which will be unpaid.

Benefits:

- Incentives will be provided in accordance with the company policy and the established incentive structure.
- Bonus will be awarded based on yearly performance and subject to eligibility and applicability criteria.
- You are eligible for Annual Leave as per the company Leave Policy post your probation.

Acceptance and Commencement:

Your appointment will be effective on your Joining Date. If you do not confirm your acceptance per the directions listed below or we are unable to set an alternative date, this offer will be withdrawn.

To confirm your acceptance to the offer, you are required to:

- Respond via email to *pooja.hr@corizo.in* to communicate acceptance of the offer and to confirm your Joining Date.
- Please note, if you do not send your acceptance to the email ID mentioned above within two (2) days of receipt of the offer, the offer will stand withdrawn.

Your employment will be contingent upon you executing an Employee Non-disclosure Agreement with the Company on the Joining Date.

5.2 Industry Overview- Global and Indian Scenario

The global education technology (EdTech) market has grown significantly in recent years due to the need for individualized and easily accessible learning options, as well as the quick development of internet access and technological advancements. According to projections, the global EdTech market may be worth billions of dollars by 2026. The market of remote study is increasing rapidly as in this virtual platform of study only requires few resources such as a good internet connection and some qualified teachers. as this means of study is easily attainable, and in students are usually free to hesitate, and they can attend the classes in flexible hours as well [19].

Edtech companies were on an exponential as during COVID-19 as well in post COVID phase. Students can use all digital equipment during or after the class which usually enhance their skills as well as improve their resume for future purposes. The virtual means of study makes students futuristic, through these edtech companies, individuals can easily prepare for different upcoming tests as well as can easily come up to learn various computer languages, as the study portal is always available for 24/7. India is having huge networks of higher education institutions in the world, with ~260 million students enrolled in >1.5 million schools and ~39,000 colleges, primarily dominated by the private sector. In India, the education market stood at US$ 100 billion in 2016 and was expected to reach US$ 180 billion by 2020[2]. Nevertheless, the edtech companies start to decline in their business models as many students start to understand the trend of the ongoing newcomers in the market as the offline study centres are easily accessible after the post-corona phase, people are somewhat not relying on these start-ups nowadays[19].

5.2.1 INTRODUCTION

CORIZO is an online learning platform where the teacher provides student training plus internships in technical, management, medical as well as in law subjects. Corizo provides various domains to the students, such as AI/ML data science, finance, human resource management, genetic engineering, nanotechnology, corporate law etc, Corizo provides 22 of certification courses for students conducted by highly educated mentors who usually have 10-15 years of experience in the educational field.

These programs make the academic base strong of the student as well as hands-on experience is also given to them as they undergo 2 months of Internship and training, where have to complete two projects one minor and one major in group as well as individually, respectively . Corizo not only makes the student bright students but also helps to enhance their leadership skills as corizo runs a internship of campus ambassador where students have to connect with their friend not from their class only but to the students from different as well to join the group where the academic counsellor add all the necessary details regarding the training and internship, Additionally, Corizo is in the official collaboration with more than 50 top MNCs where if the student do well in their internship and training their resume is circulated in the top MNCs and they can offer them a PPO from 6 LPA to 8 LPA. This collaboration helps students gain insights into what employers are looking for and prepares them to meet those expectations. Students.

Corizo provides students healthy environment along with that provide all the resources like it offers one on one session with the mentor for doubt solving sessions as well as corizo runs their live classes as per the availability of the students and also provides recorded lecture if missed live classes missed by the students.

Vision

"To provide learners and professionals with the knowledge and skills they need to succeed in today's world.".

Mission

Our mission is to empower learners and professionals with knowledge and skills that are essential in today's rapidly evolving world.

CHAPTER 6

CORIZO WORKING PROCEDURE AND METHOD

6. CORIZO WORKING PROCEDURE AND METHOD

6.1 Curriculum Development:

In order to develop and design learning content, EdTech companies hire instructional designers and subject matter experts. Videos, eBooks, cellular applications, and courses are some of the media through which this content is made available.

6.2 Technology Platform:

In order to share their pedagogical materials, they develop new technology platforms or use the existing ones. It could be developing mobile applications, learning management systems (LMS), or integrating platforms.

6.3 Marketing and Selling: EdTech businesses must approach their target audience and persuade them to sign up for their courses or programs. It includes marketing strategies like content marketing, social media marketing, and institutional collaboration.

6.4 Student Support:

The students need assistance throughout their learning process after becoming members. This may include giving them technical support, responding to questions regarding the content and giving them feedback on their performance.

6.5 Learning Experience Design: Outline the methods employed by the company to design an optimal learning experience for students. This may include user interface design, interactive elements, and personalized learning paths.

6.1.1 Curriculum Design:

Outline the curriculum design process of Corizo edtech. This can include collaborating with subject matter specialists, learning designers, and educators to create effective and interactive learning content

6.6 Teacher Training and Support: Explain how Corizo edtech enables teachers to make the most ot of their platforms. This is through training, webinars, or continued support to ensure teachers are able to utilize the technology to its full capacity

6.7 Student Engagement Strategies: Highlight the strategies employed by Corizo Edu-Tech to keep students engaged in the learning process. This may involve gamification, interactive quizzes, and other ways of boosting students' motivation and engagement.

CHAPTER 7

INTERNSHIP

7. INTERNSHIP

CORIZO provides an Industrial Training + Internship + Placement program in collaboration with 25+ MNCs. We have two kinds of programs: TRAINING & ADVANCED PROGRAMS. TRAINEESHIP: This is a 2-month Industrial training and internship placement program with Corizo. The first month will be Industrial training with practical knowledge from scratch to advanced, with 15+ years of experience, expert live interactions, and recorded backup up to 1 year. The Second Month is an internship with a partnered company where real-time projects will be assigned, including Minor & Major. Advanced Program: This is a 3-month program that includes 2 months of Industrial training for a crash course on Scratch to advance, especially for Beginners to understand each concept. Afterwards Internship with 3 real-time projects from partnered companies includes Minor and 2 Major. After the successful completion of the program, Corizo rewards 5 Major certificates to students. INTERNSHIP completion certificate powered by Digital India and the Ministry of MSME. Outstanding Performance Certificate from Corizo. Examination Completion Certificate from Corizo. LOR (Letter of Recommendation) for Job and internship opportunities. Resume building and Interview preparation sessions. Mock Interviews Online Assessments

LinkedIn Profile Maintenance Session, Personality Enhancement Session, Skill Development Session, and paid internships in MNCs based on interviews and projects. What was my key role as an intern during the internship period? As an intern in the sales and Marketing department, in my Marketing role, my primary responsibilities likely encompassed a blend of acquiring knowledge, actively participating in daily operations, and assisting the team in generating potential business opportunities.

Actively engaging in training sessions to learn sales strategies, tools, and methodologies.

- Conducting thorough research to identify potential leads and prospects for the business.
- Collaborating closely with team members in the sales department and other relevant departments.
- Calling leads and pitching them the product according to their need.
- Making a professional relationship with the client.
- Contributing to the attainment of sales targets set by the team or organization.

CHAPTER 8

LEARNING OUTCOMES AND RECOMMENDATIONS

8.1 LEARNING OUTCOMES

The internship experience was insightful where I get to know about the ongoing market trends. It got to know about how the real-world settings about corporate life, also the environment was very supporting by our seniors such as team leader manager. This collaborative environment significantly enriched my overall learning journey. Initially, I have to connect with the leads and I have to convert them into potential ones so that they can generate the resources for our sales team, these helps me to gain convincing skills as well I get a chance to enhance my communication skills but connecting with 50 -60 leads a day. I faced various failures and how to correct the mistake by looking upon the matter.

Apart from this, my education was complemented by sites such as Unstop, where I put theoretical marketing concepts into action in actual practice, enhancing my understanding of customer behaviour and the sales process. This experience has, overall, been a tremendous advantage to my career development, providing me with the appropriate tools and information required for a successful sales and marketing career.

8.2 RECOMMENDATIONS

As a relatively new entrant in the sales industry, corizo is seeking to leave its mark and plan for expansion. A framework capable of supporting the company's growth plans. In light of this need, Corizo is committed to having a formal process, including regular team meetings with managers. The meetings are designed to increase team members' awareness of each other's roles and responsibilities, thus fostering teamwork essential in expanding the business effectively.

Corizo also recognizes the values of maintaining momentum and preventing too long a period of stagnation. Operating occasional events can be used as an incentive to elicit support and enthusiasm of employees. Moreover, making the most use of holiday periods, particularly when students are more leisure time available, is a great method for the business to gain ground and grow faster.

CHAPTER 9

DISCUSSION AND CONCLUSION

9.1 DISCUSSION AND CONCLUSION

NTM is a type of mycobacteria that is present everywhere in the surroundings and may mostly infect to immunocompromised people but can also infect immunocompetent people. pulmonary diseases are the most occurring diseases, as well as skin and soft tissues. immunocompromised people are more susceptible who have some pre-existing condition of HIV, AIDS or diabetes NTM has started to increase its prevalence all over the world due to the ignorance of pathogenicity about NTM. However, *M. smegmatis* is known for its non-pathogenic nature. In the last few years, the number of cases of *M. smegmatis* has also usually caused diseases in soft tissue and skin due to some skin treatments. *M. smegmatis* due to its non-pathogenic nature, operation on this mycobacterium is easy; there is no requirement for any biosafety levels, also grows in a temperature range of 55-60°celsius.

NTM are rapidly growing mycobacteria, having very little generation time and also having the capability in any environment where they find an opportunity. *M. smegmatis* is considered the model organism for various pathogenic species in Mycobacterium because it carries more than 70 % of its related species and also has about 95% of conserved regions similar to the 90% conserved region present in *M. tuberculosis*.

The initial part of the work was done between July 2024 to December 2024, where I learned different microbiology and molecular biology techniques afterward, the second part was done as an internship in a company naming Corizo from February 2025 to April 2025, where I got a chance to know about the ongoing market trends, as well the setup of the EdTech companies.

As an Intern in corizo it was an insightful experience the never-give-up attitude of the company and always eager to do and learn new things. Corizo is one of the rapidly growing start-ups as due to their adaptation on the changing trends of the market, Corizo has two branches but they are looking to expand their branches in the northern side of India. After the completion of the internship and training, certification as well as Letter of Recommendation is given to students which open the doors for the students for their future.

Corizo provides theoretical knowledge but along with that also provide students with hands on knowledge in their respective subject they enrolled for, also provides personal assistance to the students where the session is taken to rebuild their resume, preparing them for group discussions and interviews. Bright students are also offered Pre-Placement Offers from the top MNCs. Main target of Corizo is college student where students are always seeking for some opportunities to gain some knowledge as well to enhance their resumes altogether.

REFERENCES

10 REFERENCES

[1] B. A. Brown-Elliott and J. V. Philley, 'Rapidly Growing Mycobacteria', *Microbiol. Spectr.*, vol. 5, no. 1, p. 5.1.02, Feb. 2017, doi: 10.1128/microbiolspec.TNMI7-0027-2016.

[2] L. Rigouts and S. Cogneau, 'The Genus Mycobacterium', in *Practical Handbook of Microbiology*, 4th ed., L. H. Green and E. Goldman, Eds., CRC Press, 2021, pp. 443–464. doi: 10.1201/9781003099277-35.

[3] R. Bansal, D. Sharma, and R. Singh, "Tuberculosis and its Treatment: An Overview," *Mini-Reviews in Medicinal Chemistry*, vol. 18, no. 1, Sep. 2016, doi: 10.2174/1389557516666160823160010.

[4] J. A. S. T, R. J, A. Rajan, and V. Shankar, 'Features of the biochemistry of Mycobacterium smegmatis, as a possible model for Mycobacterium tuberculosis', *J. Infect. Public Health*, vol. 13, no. 9, pp. 1255–1264, Sep. 2020, doi: 10.1016/j.jiph.2020.06.023.

[5] I. L. Sparks, K. M. Derbyshire, W. R. Jacobs, and Y. S. Morita, 'Mycobacterium smegmatis: The Vanguard of Mycobacterial Research', *J. Bacteriol.*, vol. 205, no. 1, pp. e00337-22, Jan. 2023, doi: 10.1128/jb.00337-22.

[6] R. Bansal, D. Sharma, and R. Singh, 'Tuberculosis and its Treatment: An Overview', *Mini-Rev. Med. Chem.*, vol. 18, no. 1, Dec. 2017, doi: 10.2174/1389557516666160823160010.

[7] J. M. Grange and A. Zumla, 'The global emergency of tuberculosis: what is the cause?', *J. R. Soc. Promot. Health*, vol. 122, no. 2, pp. 78–81, Jun. 2002, doi: 10.1177/146642400212200206.

[8] S. Chetty, M. Ramesh, A. Singh-Pillay, and M. E. S. Soliman, "Recent advancements in the development of anti-tuberculosis drugs," *Bioorganic and Medicinal Chemistry Letters*, vol. 27, no. 3. Elsevier Ltd, pp. 370–386, 2017. doi: 10.1016/j.bmcl.2016.11.084.

[9] M. Druszczyńska, M. Kowalewicz-Kulbat, M. Fol, M. Włodarczyk, and W. Rudnicka, 'Latent Mycobacterium tuberculosis Infection – Pathogenesis, Diagnosis, Treatment and Prevention Strategies', *Pol. J. Microbiol.*, vol. 61, no. 1, pp. 3–10, 2012, doi: 10.33073/pjm-2012-001.

[10] K. To, R. Cao, A. Yegiazaryan, J. Owens, and V. Venketaraman, 'General Overview of Nontuberculous Mycobacteria Opportunistic Pathogens: Mycobacterium avium and Mycobacterium abscessus', *J. Clin. Med.*, vol. 9, no. 8, p. 2541, Aug. 2020, doi: 10.3390/jcm9082541.

[11] J. T. Crawford and J. H. Bates, 'Isolation of plasmids from mycobacteria', *Infect. Immun.*, vol. 24, no. 3, pp. 979–981, Jun. 1979, doi: 10.1128/iai.24.3.979-981.1979.

[12] J. Trias and R. Benz, 'Permeability of the cell wall of *Mycobacterium smegmatis*', *Mol. Microbiol.*, vol. 14, no. 2, pp. 283–290, Oct. 1994, doi: 10.1111/j.1365 2958.1994.tb01289.x.

[13] Z. He and J. De Buck, 'Cell wall proteome analysis of Mycobacterium smegmatis strain MC2 155', *BMC Microbiol.*, vol. 10, no. 1, p. 121, 2010, doi: 10.1186/1471-2180-10-121.

[14] J. A. Newton, P. J. Weiss, W. A. Bowler, and E. C. Oldfield, 'Soft-Tissue Infection Due to Mycobacterium smegmatis: Report of Two Cases', *Clin. Infect. Dis.*, vol. 16, no. 4, pp. 531–533, Apr. 1993, doi: 10.1093/clind/16.4.531.

[15] C. J. Wang, Y. Song, T. Li, J. Hu, X. Chen, and H. Li, 'Mycobacterium smegmatis Skin Infection Following Cosmetic Procedures: Report of Two Cases', *Clin. Cosmet. Investig. Dermatol.*, vol. Volume 15, pp. 535–540, Mar. 2022, doi: 10.2147/CCID.S359010.

[16] C. A. Best and T. J. Best, '*Mycobacterium Smegmatis* Infection of the Hand', *HAND*, vol. 4, no. 2, pp. 165–166, Jun. 2009, doi: 10.1007/s11552-008-9147-6.

[17] J. P. Richards and A. K. Ojha, 'Mycobacterial Biofilms', *Microbiol. Spectr.*, vol. 2, no. 5, p. 2.5.16, Sep. 2014, doi: 10.1128/microbiolspec.MGM2-0004-2013.

[18] A. Gordillo-Galeano, L. F. Ospina-Giraldo, and C. E. Mora-Huertas, 'Lipid nanoparticles with improved biopharmaceutical attributes for tuberculosis treatment', *Int. J. Pharm.*, vol. 596, p. 120321, Mar. 2021, doi: 10.1016/j.ijpharm.2021.120321.

[19] A. Bansal, S. Pophalkar, and C. J. Vidani, 'A Review of Ed-Tech Sector in India'.

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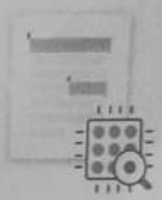

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STUDIES ON *MYCOBACTERIUM SMEGMATIS* BIOFILM AND ITS INHIBITION

Dissertation submitted in partial fulfillment of the requirement for the degree of

Master of Science

In

Biotechnology

By

Avek Singh Halla (235111040)

Under the supervision

of

Dr. Rahul Shrivastava

To

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