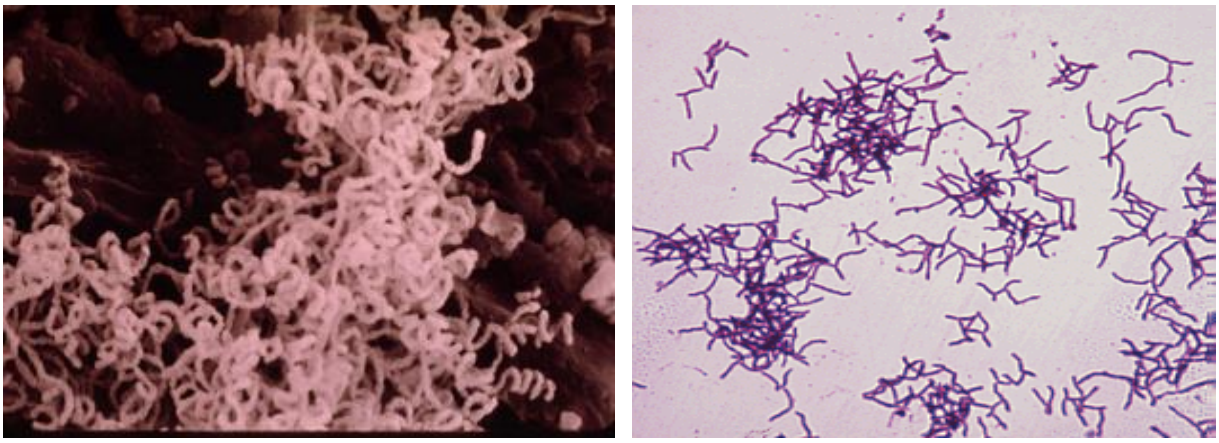
Practical Microbial Metabolism // 4th Stage **Lab 2**
Isolation of an antibiotic producer (Actinomycetes) from soil **4 pages**

Introduction

- Soil is the major repository of microorganisms that produce antibiotics capable of inhibiting the growth of other microorganisms. Clinically useful antibiotics have been isolated from several groups of soil microorganisms, including bacteria (*Streptomyces spp., Bacillus spp.*) and fungi (*Penicillium spp. Cephalosporium spp.*)
- Although many organisms in soil produce antibiotics, only a small portion of new antibiotics is suitable for medical use.
- Also, pharmaceutical companies directing its energies toward chemical modification of existing antibiotic substances.
- This is accomplished by adding or replacing chemical side chains, or by reorganizing intramolecular bonding, or by producing mutant microbial strains capable of excreting a more potent form of the antibiotic.
- These chemically modified antibiotics have been responsible for the prevention of antibiotic resistance, minimizing the side effects in the host, and increasing the effective spectrum of a given antibiotic.

General characteristic of Actinomycetes

1. Actinomycetes are similar to both bacteria and fungi, and have characteristics linking them to both groups.
2. Gram-positive, endospore forming bacteria but produce a mycelium which is non-septate.
3. They are mostly aerobic organisms, but some can be anaerobic as well.
4. On an agar plate inoculated with soil, numerous Streptomyces colonies develop, the colonies are easy to recognize by their pastel colors and characteristic texture (a hard mass that extends into the agar).
5. Some of the filaments grow into the agar. The rest extend up from the agar and form chains of reproductive asexual spores at the tips.
6. Reproduction is through hyphae fragmentation or spore formation.
7. Although their general growth pattern is fungus-like, actinomycetes are distinctly prokaryotic in size and structure. In cross section an actinomycete mycelium looks like a typical Gram-positive bacterial cell.
8. Some types of Actinomycetes are responsible for the peculiar odor emanating from the soil after rain, mainly in warmer climates.



Morphology and Gram staining of Actinomyces

Pastel colors of Actinomyces on culture media

Importance of Actinomycetes

1. Playing an important role in decomposition of organic materials, such as cellulose, chitin, proteins and fats.
2. They are also responsible for producing more than 70% of all currently known antibiotics. The antibiotic substances they produce display antibacterial, antifungal, antitumor, antiprotozoic and antiviral properties.
3. Producing geosmin, the gaseous compound which gives soil a characteristic earthy odor.
4. Capable of biological nitrogen fixation with *Frankia* species.

***Streptomyces* spp**

- Streptomyces is the most common actinomycete genus in soils, which forms 90 % of the populations.
- Most *Streptomyces* species are aerobic, psychrophilic, or mesophilic saprophytes that are frequently found in soil.
- Thermophilic *Streptomyces* have an optimum temperature above 50 C such as S. thermophilus.
- Unlike most other eubacteria, chromosomes of *Streptomyces* are linear.
- One of the most important properties of Streptomyces is their capacity to produce antibiotics. More than 500 antibiotics are known to be produced by Streptomyces.
- The antibiotics produced by *Streptomyces* are typical secondary metabolites produced toward the end of the exponential phase of growth.

Some common antibiotics synthesized by *Streptomyces* spp

Common name	*Producer organism*	*Antibiotic spectrum*
Streptomycin	*S. griseus*	Most Gram-negative bacteria
Spectinomycin	*Streptomyces* spp.	*M. tuberculosis, N. gonorrhoeae*
Neomycin	*S. fradiae*	Broad spectrum (topical application)
Tetracycline	*S. aureofaciens, S. rimosus*	Broad spectrum
Chlortetracycline	*S. aureofaciens*	Broad spectrum
Erythromycin	*S. erythreus*	Frequently used in place of penicillin
Lincomycin	*S. lincolnensis*	Obligate anaerobes
Nystatin	*S. noursei*	Antifungal
Chloramphenicol	*S. venezuelae*	Broad spectrum
Amphotericin B	*S. nodosus*	Antifungal

Factors influencing actinomycetes population in soil:

- Organic matter
- pH
- Temperature
- Moisture

Procedure:

There are three major steps for isolation of Actinomycetes from soil as follow:

First step: Primary isolation of Actinomycetes.

Second step: Colony Selection and Inoculation.

Third step: Evidence of Antibiosis and confirmation.

First step: Primary isolation of Actinomycetes

Materials:

- Soil sample
- Test tubes
- Glycerol yeast extract agar plates
- Pipettes
- Cotton swabs or L-shape glass rod

Procedure:

1. Label six test tubes from 1 to 6 with a wax pencil.
2. Using a 10ml pipette, dispense 9 ml of saline into each tube.
3. Weigh out 1g of soil and deposit it into tube 1.
4. Mix tube 1 until all soil is well dispersed throughout the tube, mix for at least 1 minute.
5. Make a tenfold dilution from tube 1 to tube 6 by transferring 1ml of soil mixture from tube to tube.
6. Label three glycerol yeast extract agar plates with your initials, and the following dilutions: 1:10,000 (10^{-4}), 1:100,000 (10^{-5}), and 1:1,000,000 (10^{-6}).
7. Transfer 0.1ml from the soil mixture in tubes 4, 5, and 6 to the appropriate plate.
8. Spread the organisms over the agar using the spreader.
9. Incubate the plates at 30° C for 7 days.