# Plant tissue culture techniques and sterilization

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محاضرة 2

# Sterilization

Killing or excluding microorganisms or their spores with heat, filters, chemicals or other sterilants

## Tissue culture is an aseptic technique

Aseptic technique:

- Sterile
- Free of pathogenic microorganisms
- Free or freed from pathogenic microorganisms
- Free from the living germs of disease and fermentation
- Conditions established to exclude contaminants

# Sterilization
# the growth of contam cause

1. Micro-organism contamination can over grow the plant culture resulting in culture death
2. Micro-organism contamination exhaust the nutrient media
3. Micro-organism can change in secondary metabolite structure or produce other compounds .

# Source of contamination

The explant or culture ✓

The vessels ✓

The media ✓

The instruments ✓

The environment where handling is taking place ✓

# Aseptic Techniques

✓Chemical treatments

- disinfectants,
- antibiotics,
- sublimat

✓Physical treatments

- heating: the most important disinfection method
- electromagnetic radiation,
- filtration
- ultrasonic waves.

# Disinfectans

- ✓They penetrate into bacteria,
- ✓They will denature bacterial protein,
- ✓They decrease the activity of bacterial enzyme,
- ✓They inhibit bacterial growth and metabolism,
- ✓They damage the structure of cell membrane,
- ✓They change membrane permeability.

# Disinfectans

- Liquid laundry bleach (NaOCl at 5-6% by vol)
  - Rinse thoroughly after treatment
  - Usually diluted 5-20% v/v in water; 10% is most common
- Calcium hypochlorite – $Ca(OCl)_2$
  - a powder; must be mixed up fresh each time
- Ethanol (EtOH)
  - 95% used for disinfesting plant tissues
  - Kills by dehydration
  - Usually used at short time intervals (10 sec – 1 min)
  - 70% used to disinfest work surfaces, worker hands
- Isopropyl alcohol (rubbing alcohol) is sometimes recommended

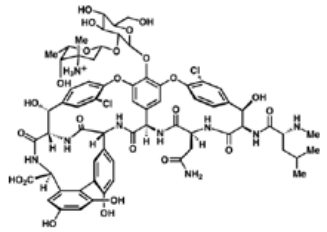
# Antibiotics

- ✓Used only when necessary or when disinfestants are ineffective or impractical
- ✓Its use by incorporating in the media
- ✓Common antibiotics are carbenicillin, cefotaxime, rifampicin, tetracycline, streptomycin
- ✓Problems with antibiotics
  - tend to be selective
  - resistance acquisition
  - may obscure presence of microbes
  - cell/tissue growth inhibition

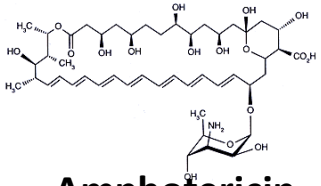
# An ideal antibiotics

- ✓ Broad-spectrum
- ✓ Did not induce resistance
- ✓ Selective toxicity, low side effects
- ✓ Preserve normal microbial flora

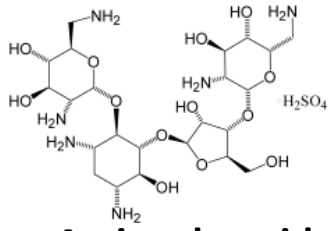
# Modes of action



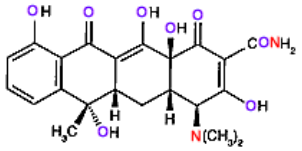
vancomycin



Amphotericin



Aminoglycosides



Tetracyclines

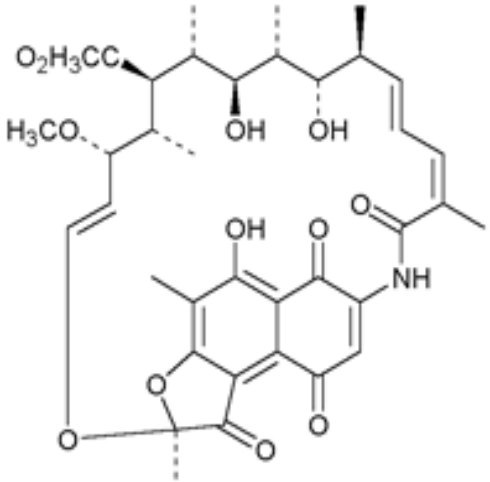
- **Inhibitors of cell wall synthesis**.
  Penicillins, cephalosporin, bacitracin, carbapenems and vancomycin.
- **Inhibitors of Cell Membrane.**
  Polyenes - Amphotericin B, nystatin, and condicidin.
  Imidazole - Miconazole, ketoconazole and clotrimazole.
  Polymixin E and B.
- **Inhibitors of Protein Synthesis.**
  Aminoglycosides - Streptomycin, gentamicin, neomycin and kanamycin.
  Tetracyclines - Chlortetracycline, oxytetracycline, doxycycline and minocycline.
  Erythromycin, lincomycin, chloramphenicol and clindamycin.

# Modes of action

- **Inhibitors of metabolites (Antimetabolites).**

  Sulfonamides - Sulfanilamide, sulfadiazine silver and sulfamethoxazole.

  Trimethoprim, ethambutol, isoniazid.




**rifamycin**

- **Inhibitors of nucleic acids (DNA/RNA polymerase).**

  Quinolones - Nalidixic acid, norfloxacin and ciprofloxacin.

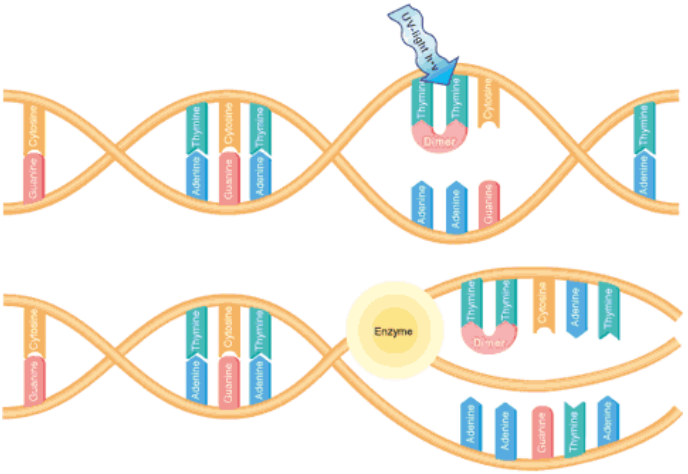
  Rifamycin and flucytosine.

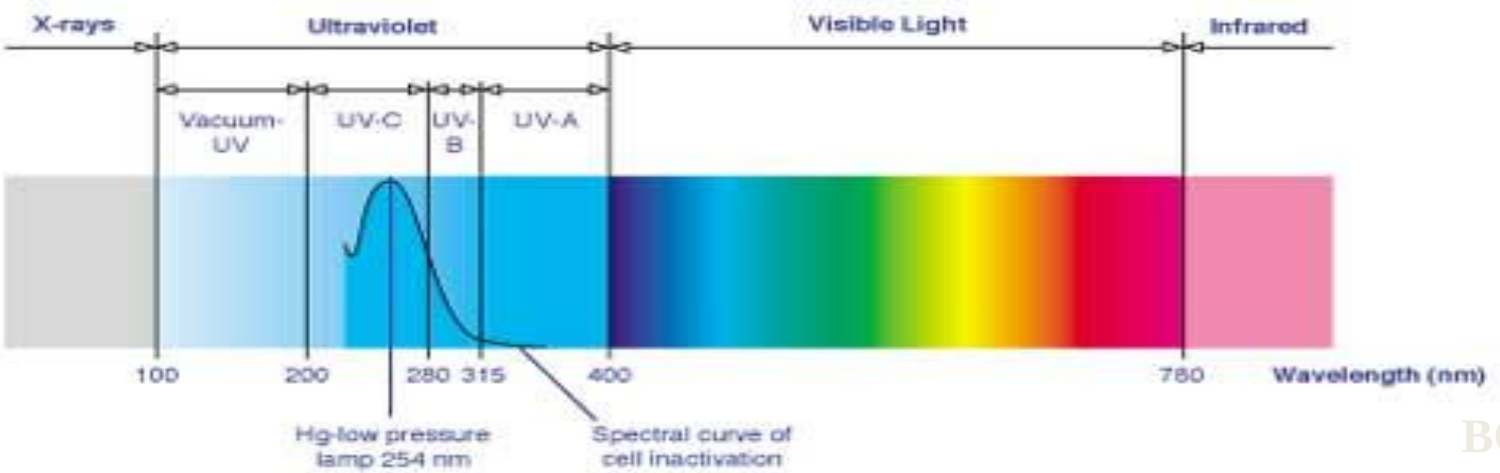
# Sublimat (0.1 - 1%)

- ✓Its activity based on $Cl^-$
- ✓Heavy metal (Hg) denaturates proteins.
- ✓Hg is toxic for the environment, therefore recuperate the Hg-solution after use and collect in a large container.
- ✓ Hg can be precipitated by adding ammonia to the solution, and siphoning the supernatant

# UV radiation

- Ultraviolet is light with very high energy levels and a wavelength of 200-400 nm.
- One of the most effective wavelengths for disinfection is that of 254 nm.



# Heating

- Oven (dry heat)

  Suitable for tools, containers a 160°-180° C for 3 h

- Microwaves (off the shelf)

  Useful for melting agar (but not gellan gum types of solidifying agents)

  Special pressurized containers are required for sterilizing in a microwave

- Flaming or heating of tools

  Flaming – e.g., 95% EtOH in an alcohol burner is useful for sterilizing metal instruments

  Bacticinerators – heats metal tools in a hot ceramic core

  Heated glass beads

# Heating

- Autoclave

  Steam heat under pressure (It typically generates 15 lbs/in$^2$ and 250° F (1.1 kg/cm$^2$ and 121° C))

  It is faster and more effective

  For liquids (such as water, medium), autoclave time depends on liquid volume

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# Filtration

- Filtration of culture medium
  - Some medium ingredients are heat labile, e.g., GA, IAA, all proteins, antibiotics
  - Most devices use a paper cellulose filter with small pore spaces (0.22 µm)
  - Syringes used for small volumes, vacuum filtration for large volumes
- Filtration of air
  - Transfer hoods usu. generate wind at 27-30 linear m per min (or 90-100 ft per min)
  - Too slow and air drops contaminants onto your work surface; too fast causes turbulence and excess filter wear
  - air "corridors" must be kept free of barriers to be effective