

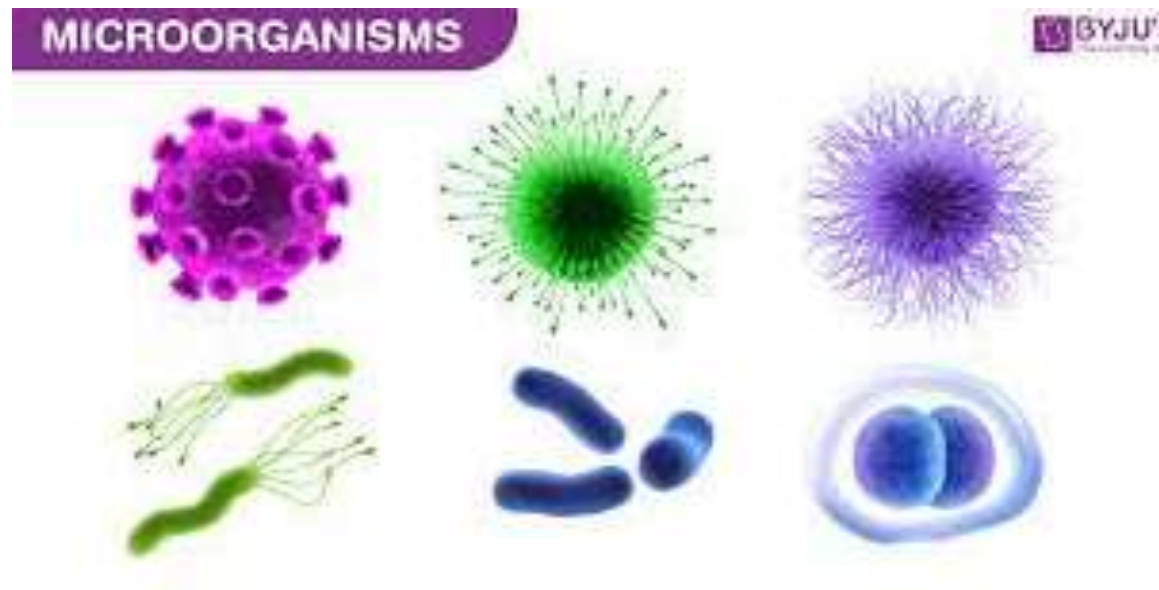
Université Abou Bekr Belkaïd – Tlemcen-  
Faculté des Sciences de la Nature et de la Vie,  
Sciences, de la Terre et de l'Univers  
Département de Biologie




Master 2 Microbiologie et contrôle  
de qualité

By Dr. Hamza Reguig Sherazad

- **Microbiology**, the study of single-celled microscopic life.
- Also, microbiology is critical in today's food, medical, and biotechnical industries.




- 
- Since microbes are everywhere on our hands, our clothes, and our laboratory work surfaces a problem with many microbiology studies in the classroom or school lab is the risk of contamination of cultures by unwanted species

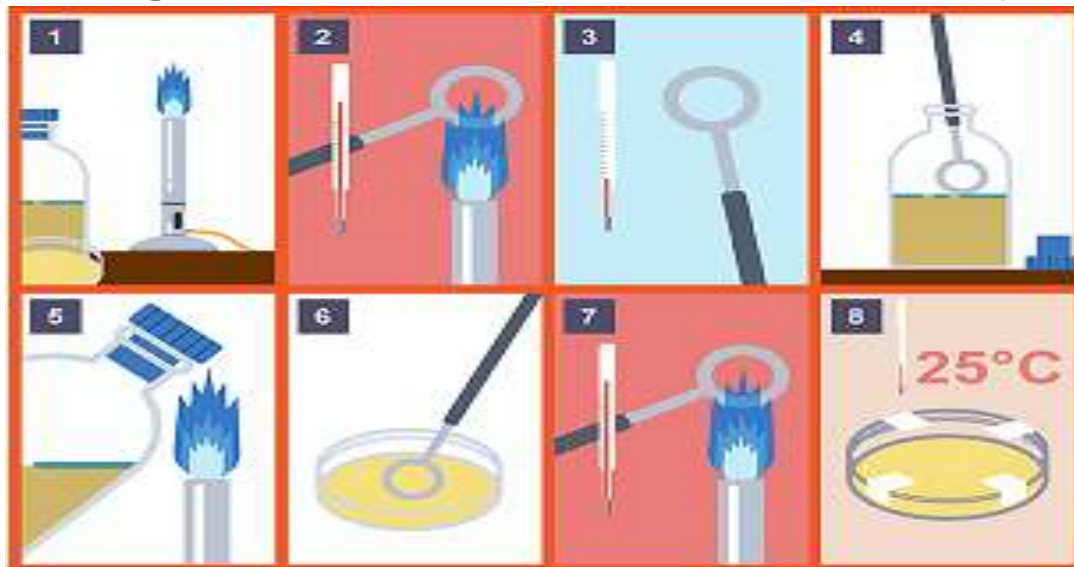
- Avoiding contamination is everyday work for those who use microbiology in their professions



- In medicine, researchers and technicians grow microbes from sick patients in order to identify the **pathogen** (disease-causing agent), or to test a pathogen's antibiotic resistance.

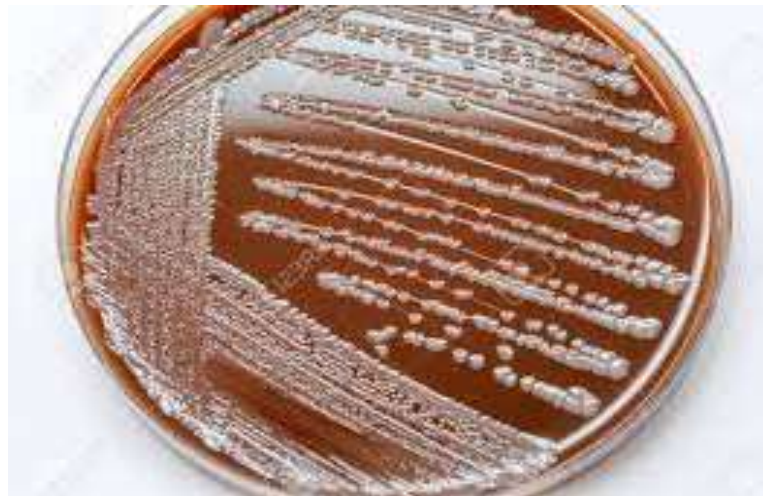
- 
- This work with known or potential pathogens requires special laboratory procedures for handling, containment, and disposal.
  - These procedures are also used in the food and biotechnology industries, where workers carefully monitor microbe strains and populations (e.g., the milk supply is regularly tested for harmful microbes.)


To deal with the problem of contamination, microbiologists follow a basic set of procedures known as **sterile** or **aseptic technique**. Sterile technique is used for culturing and transferring cultures, and for streaking plates to isolate and purify strains.






- While learning sterile technique, another important microbiology skill is plate streaking.
- The purpose of streaking agar plates is to generate individual colonies for examination or study.



- 
- The technique is also used to isolate individual microbial species from a mixture of microbes in a sample.
  - In order to generate single colonies on a plate by streaking, you must have good streaking technique.





- 
- Consider that an inoculating loop dipped into a pure culture of *Halobacterium* may pick up 10,000,000 cells. If a plate is streaked correctly, some of these cells will be spread out enough to grow individual colonies.

- *Halobacterium* sp. NRC-1 is a member of the **Archaea** and grows in extremely salty environments including salt marshes, hypersaline lakes (e.g., the Great Salt Lake)



- *Halobacterium* is grown on medium so salty that few other organisms would survive it. In fact, *Halobacterium* will **lyse** at total **salinities** below 1.0–2.0 M. (For comparison, sea water has a total salinity of 0.6 M, while human blood contains only 0.14 M NaCl)


- 
- For these reasons and the fact that *Halobacterium* is not known to cause any human disease, these microbes are ideal for beginning practice of sterile technique. Using these microbes allows students to practice the hand motions of sterile technique without the concern that they will culture a pathogen or contaminate their workspace with the microbe they are using.

- 
- **It is essential to learn good sterile technique and use the same precautions as if working with a known pathogen.**

# Materials


- *Included in the kit*
- 40 petri dishes
- 8 bottles *Halobacterium* Agar, 135 mL each
- *Halobacterium* liquid culture
- 128 sterile inoculating loops
- autoclavable disposal bag
- hand lenses, or dissecting microscope(s)
- wax markers or lab pens
- Bunsen burners
- resealable plastic bags or plastic food-storage boxes
- \*Incubator, 42° or 37°





- 
- Disinfectant for benchtop (e.g., alcohol or bleach solution)
  - If an incubator is not available, the plates may be stored on the lab bench.
  - If the plates are incubated at 42°C, results may be obtained in 7 days, and if at 37°C, 7–14 days. If the plates are incubated at room temperature on the bench, it may take 2 weeks or longer to see the results, depending on the temperature of your lab.


# Preparing Media Plates

- I. To melt the medium, slightly loosen the cap(s) and set the bottle(s) of medium in a pot of water and bring it to a boil. Make sure the water level is even with the level of the medium in the bottle(s). Leave the bottle in the boiling water until the medium has completely melted. This will take approximately 30 minutes.


- 
- **2.** Allow the medium to cool to 55°C either by allowing the pot of water to cool to that temperature or by letting the bottle(s) sit for several minutes at room temperature. The bottle(s) should feel comfortably hot to the touch when around 55°C.

- 
- **3.** Disinfect the work surface. Wash your hands thoroughly. Unpack the petri dishes, being careful not to disturb their sterility. Align the sterile plates along the edge of a clean, level tabletop away from any draft or breeze.

- 
- **4.** Remove the cap and flame the mouth of a bottle of medium. Lift the lid of a petri plate just enough to pour in the molten medium. Carefully, pour to a depth of about 5 mm per plate. Replace the lid immediately to prevent contamination.

- 
- **5.** Repeat Step 4 with the other bottle(s) of medium.
  - **6.** Let the petri plates stand undisturbed until they solidify (about 1 hour). Let the plates sit out until any condensation on the lid evaporates.



- 
- **7.** Dispose of the empty bottles in an autoclave disposal bag.

## Examination of Colonies and Explanation of Results

- Have your students compare results with each other and then draw conclusions about whether they would use the same or a different streaking technique the next time they do the exercise. In addition, have your students watch for the development of the following
- three possible **phenotypes** (all represented on the plate shown on the cover of this manual):

- •Vac<sup>+</sup>, or wild-type, pink colonies. In the cells in these colonies, *Halobacterium*'s red pigmentation is somewhat altered by the presence of gas vesicles, internal organelles that refract light and enable the cells to float in a liquid culture. *Halobacterium* converts light energy to chemical energy. Thus, this ability to float is advantageous, because it allows
- *Halobacterium* cells to float near the surface of natural lakes and ponds where the light is most intense.
- •Vac<sup>-</sup>, or red colonies. *Halobacterium*'s red color is due to its pigments **carotenoids** and **bacteriorhodopsin**. Bacteriorhodopsin enables the cells to convert light energy to chemical energy in a method similar to, but different from photosynthesis.