Lab.3: Detection of bacteriocin production by lactic acid bacteria (LAB)

Antimicrobial peptides (AMPs) are produced by eukaryotes and prokaryotes and serve as important components of their defense against microorganisms. AMPs produced by bacteria known as Bacteriocins.
Bacteriocins are biologically active proteins or protein complexes that display antimicrobial action towards usually closely related species.
Bacteriocins of lactic acid bacteria (LAB) are the most important one in biotechnology, because they may be developed into new and useful antimicrobial additives and drugs. Nisin, produced by *Lactococcus lactis* strains, is the only one with widespread commercial use as biopreservative in foods.

There are 4 methods for detection of bacteriocin production:

- **The Well-Diffusion method**

1. Inoculate 10 ml of MRS broth with 0.2 ml of LAB.
2. Incubate at 37°C for 24 hours in anaerobic conditions.
3. Centrifuge the bacterial culture at 6000 rpm for 15 minutes.
4. Spread 0.1 ml of indicator bacteria (pathogenic bacteria) on the nutrient agar plates.
5. Cut 5 wells with 6mm sterile cork-borer, fill with 100 µl of the culture supernatant.
6. Incubate the plates at 37°C for 24 hours.
7. Measure the inhibition zones and record the results. Inhibition is detected by a zone of clearing around the supernatant well.

- **The Disk-Diffusion Method**

1. Inoculate 10 ml of MRS broth with 0.2 ml of LAB.
2. Incubate at 37°C for 24hours in anaerobic conditions.
3. Centrifuge the bacterial culture at 6000 rpm for 15 minutes.
4. Spread 0.1 ml of indicator bacteria (pathogenic bacteria) on the nutrient agar plates.
5. Submerse 6mm sterile disks of filter paper in the culture supernatant.
6. By sterile forceps, place the submersed disks on the inoculated plates.
7. Incubate the plates at 37°C for 24 hours.
8. Measure the inhibition zones and record the results. Inhibition is detected by a zone of clearing around the disk.

- **The Flip-Streak Method**

1. Streak the LAB across the surface of Modified Trypton Soya Agar (MTSA) plates.
2. Incubate at 37°C for 24hours in anaerobic conditions.
3. After incubation, flip the agar onto Petri dish cover with a sterile spatula.
4. Streak indicator bacteria (pathogenic bacteria) across the surface of the agar perpendicularly to the producer streaks.
5. Incubate the plates at 37°C for 24 hours. Bacteriocin production is detected by a zone of inhibition at the intersection of the producer and indicator isolates.

- **The Spot-on-the-Lawn Method**

1. Spot Petri plates containing MTSA with 2µl of the LAB.
2. Incubate at 37°C for 24hours in anaerobic conditions.
3. Melt NA agar (9 ml in each tube) to 45°C, inoculate with indicator bacteria (pathogenic bacteria) and mix well.
4. Pour the inoculated tubes on the spotted plates.
5. Incubate the plates at 37°C for 24 hours.
6. Measure the inhibition zones and record the results. Inhibition is detected by a zone of clearing around the producer colony.