

Experiment #1. Isolation *Lactobacillus* spp. bacterial strain

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This experiment introduces the isolation of *Lactobacillus* spp. bacterial strain from yogurt purchased in the supermarket. Some *Lactobacillus* bacterial strains that live in the gut of human body, for example, *Lactobacillus reuteri*, may upregulate the oxytocin,¹ a hormone which plays a role in social bonding, and sexual reproduction. To prepare 150 ml of medium we will need:

Equipment:

- A pot;
- An electric stove;
- Two flasks;
- Petri dishes - 5 plates;
- A pressure cooker;
- An incubator;
- A pipette and tips;
- A paper clip or an alternative;
- Some alcohol for sterilization.

Ingredients:

- Yogurt with *Lactobacillus* spp. bacteria;
- MRS broth 7,8 g.;
- Agar 2 g;
- Distilled water 200 ml.

1. Suspend MRS powder in 150 ml of distilled water. Divide the solution into two flasks, one with 100 ml for the use with agar medium and another one with 50 ml for the liquid medium.
2. Add 2 g of agar to the flask with 100 ml of distilled water. To ensure homogeneity shake well the supplement before use
3. Mix well and heat with frequent agitation until the medium boils well.
4. Dispense into a flask and sterilize by cooking in a pressure cooker for 15 minutes. At the same time sterilize the liquid MRS broth.
5. Cooldown a bit the medium with agar so you could keep your hands on the flask, and pour into your both Petri dishes, each approximately 5 mm, and leave until the medium stiff.
6. Add about 1 ml of yogurt into 10 ml of sterile water. Be sure that the water is no warmer than body temperature. Shake well.
7. Take a drop of diluted yogurt with a pipette and release on the first stiffed petri dish. distribute evenly. Dilute the diluted yogurt several times, and repeat the action for the remaining Petri dishes.
8. Leave in an incubator with 37 degrees of Celsius for 2-3 days until you see the formed bacterial colonies.
9. Inspect your bacterial colonies. Take a sample of *Lactobacillus* and transfer to a fresh Petri dish with the stiffed medium. Use a sterile paper clip to distribute around the Petri dish. Leave for 2-3 days until the colonies are formed.
10. Take some biomass of the grown bacterial colonies and put them into the liquid MRS broth. Leave bacteria in the incubator with 37 degrees Celsius for a couple of days to colonize the medium.
11. Store the liquid MRS broth with the bacterial strains in a fridge at 2-8 °C.

1 Bernard J. Varian et al (2017) "Microbial lysate upregulates host oxytocin" in *Brain Behav Immun.* 2017 March ; 61: 36–49. doi:10.1016/j.bbi.2016.11.002.