BACTERIAL CROWDING – UPO Sevilla

QUALITATIVE | **CAPILLARY ASSAY USING MICROSCOPE**

1. ***E. Coli*** **inocula in triptone broth in low shaking (between 100-200 rpm/min)**

   Inocula must be incubated at low temperature and low shaking in order to achieve a peak development of flagella. High shaking might provoke the loss of flagella; the production of flagella wouldn't be possible in rich environments since bacteria wouldn't need them.

2. **Taking a sample from one inoculum and looking at the motility under the microscope.**

   It is crucial to observe that flagella have been properly developed in the bacteria; in the same way it is also important to see that those bacteria are mobiles. If the final result is a low motility, the assay should be restarted.

3. **Diluting the** ***E. Coli*** **culture 100 times.**

   We dilute the triptone broth in E. Coli culture a hundred of times. Incubate again at 30ºC in low shaking till exponential medium phase. The final volume would be 20 ml so we will have to inoculate 200 μl.

4. **Check motility under the microscope. Again, we check the motility to observe that it still exits.**

5. **Wash twice the culture centrifugating and resuspending in chemotaxis buffer.**

   It's essential to be really careful when resuspending pellets, it is needed to hit softly in the base of the tube. The centrifuges must be done in a low speed to prevent the loss of flagella (10 min in 8000 G)

6. **Measure optical density and adjust the volume in order to achieve around 10^7 cfu/ml (optical density ~0,01).**

1. **Set up the assay**

   Two capillaries are put over a microscope slide which will hold up a cover slip. Then we insert the bacterial dilution between the slide and the cover slip. Two new capillaries are inserted between the slide and the cover slip, inside of the bacterial dilution. One of those capillaries would contain a chemoattractant while the other one would be the control. Under a microscope we could see the difference between both capillaries and we would definitely be able to observe if there is chemotaxis toward this chemoattractant. The capillary located at the end is heated sealed meanwhile the chemo attractant is sucked.

   It's possible to look at the microscope slide under the microscope in different times and observe the diverse quantity of bacteria in the chemotactic capillary and in the control capillary.

8. Dilution and spread (optional)

After the incubation, the quantity of bacteria in those capillaries might be quantified when spread in plates. For that it is necessary to empty previously the capillary by means of centrifugation: The capillary content is obtained in a 1.5mL tube by centrifugation: Wash the outside of capillaries using distilled water and then break the seal and empty capillaries into 1.5mL tubes that contain 200 µl of chemotaxis buffer; after that , centrifuge it.

IMPORTANT: To work with *Pseudomonas* it is necessary to set up inocula in minimal medium instead of triptone broth. Plus, the culture must rest till it achieves the exponential late phase, since it is in here when flagella are developed. At the end of this assay the final incubation must be done at 30ºC.