BACTERIAL CROWDING – UPO Sevilla

QUANTITATIVE | **CAPILLARY ASSAY USING 96-WELL PVC MICROPLATES**

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).**

7. **Assay set-up**

1 µl capillary pipettes would be used as a capillary. One of its ends will be heat sealed. At the same time, the unsealed end will suck the chemo attractant; Whereas the control capillary won't suck the chemo attractant but chemotaxtis buffer.

As for the chemotactic chamber, 96-well PVC microplates will be used. Each well will be filled with some bacterial dilution of 200µL. In order to maintain capillaries still agarose gel 2% will be used; that is to say, this gel will be put above

the 96-well PVC micro plate and will be pierced by the capillaries.

8. Incubation at 30ºC for 1h.

9. Dilution and spread in LB plates.

Once the capillaries have been taken out from the gel, the dilution and spread in LB plates is started. 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. LB plates are incubated overnight.

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.