BACTERIAL CROWDING – UPO Sevilla

QUANTITATIVE

Capillary Assay in tip chambers

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. **Setting up the assay.**

   We spread 22mL of the bacteria dilution in each well in a tip empty sterilized chamber. Aside, we take a sample of inocula and dilute it 10^5 times and spread it in order to quantify the number of bacteria. Once the chemotactic chamber is ready, we place some syringe's needles in each cubicle filled with either the chemoattractant or the repellent we want to study, plus the appropriate control at 30ºC. Leaving the chemotactic chamber as horizontal and immobile as possible.

8. **Dilution and spread in LB plates using the content of capillaries**

   Dilute the content of capillaries (we use 2 10^2 and 2 10^4 dilution factor). Spread in LB plates and incubate it at 37ºC overnight. Also, you could quantify bacteria which are inside of capillaries by fluorescent microscope supposing those

bacteria have any fluorophore.

Important. On the one hand, for this assay, we have been using two different kinds of capillaries, both syringe's needles and micropipettes tips. On the other hand, we have also used two diverse types of chamber, principally and more often tip chamber, but occasionally needle's cups or heated sealed 10μl tips.
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.