Bacterial Growth

1) Bacterial growth on agar - This is simplest protocol to conduct in the laboratory. In brief, the students can inoculate a single colony of their chosen organism onto agar plates and incubate the plates overnight (ON). The incubation conditions can be varied, students can incubate the plates aerobically or anaerobically (you need an anaerobic jar for this), they can vary the temperatures and even the agar used. Some agars are differential, in that different types of bacteria grow differently on the agar and can be distinguished from one another, other types of agar are selective and can be used to promote the growth of one organism while inhibiting the growth of another. The class example that students could use here is MacConkey agar that selects for gram-negatives through the addition of bile salts (organisms adapted to the gut readily survive bile) whereas gram-positive bacteria succumb. In addition pH indicators allow students to identify lactose versus non-lactose fermenters (lactose fermenters grow as bright pink colonies) [1]. Another important feature of agar is that it will always be used when plating for counts is needed. To plate for counts, serial dilutions of bacterial suspensions are spread over the surface of the agar plates and they are incubated overnight. The resulting colonies that form are converted into counts by multiplying by the dilution factor [2-3].

2) Bacterial growth in broth - In brief, the students can inoculate a single colony of their chosen organism into 2 ml broth and the broth incubated overnight. In the morning, the amount of cells is estimated by measuring the turbidity (this is done using standards such as McFarland Standards which can be purchased from scientific supply companies) or optical density (which is done using a spectrophotometer to measure the OD600 nm (with plating for counts)). Broth, like agar, can be varied in composition and the conditions used to incubate can vary such as temperature and aeration (shaking increases the oxygenation of the culture).

3) A growth curve - This can be done on agar or in broth [4]. A fixed amount of the ON culture is added to agar or broth and the samples incubated. For agar, this would mean several identical plates would be incubated and one sacrificed at each sampling time point (Bacterial growth would be estimated by removing the bacteria from the surface of the agar with a sterile spreader, diluting and spreading for counts). For the broth samples, aliquots of the culture would be removed with a pipette, diluted and the dilutions spread onto agar. Samples would be taken at several time points over the course of the lab, (for *E. coli* this usually in 20 min periods) and the OD600 nm recorded with the spectrophotometer, and the cells diluted and plated for counts on agar. Bacterial growth curves for *E. coli* can be completed in about 25 hrs with the bacteria reaching stationary phase in about 6 hrs.

4) Continuous bacterial growth - Growing bacteria in a continuous culture requires a chemostat or bioreactor, which is not a common piece of laboratory equipment. A virtual bioreactor is available for students to learn about the principals [5].

5) Growth in fermentation tubes - Bacteria can be grown in fermentation tubes [6]. In brief, a colony of the chosen bacteria is used to inoculate a sugar tube with an inverted Durham tube. It is incubated overnight and interpreted the following morning. The sugars used are generally glucose, sucrose and lactose. If the organism is able to ferment the sugar, a pH indicator in the broth will demonstrate this process by changing color. If the organisms are able to produce gas, this will manifest as a bubble in the tube. This assay could be modified to include testing the pH over time using pH indicator strips [7]. The change in pH could be recorded by students and plotted on a graph (time versus pH).

6) Growth in Triple sugar iron (TSI) slants - A modification of the fermentation tubes, this involves an agar slant that is stabbed and streaked and incubated [8]. It is a tubed differential medium that can determine carbohydrate fermentation, gas production as well as H_2S production. Bacteria can metabolize carbohydrates (sucrose, lactose and glucose) aerobically (with oxygen) or by fermentative processes (without oxygen).

7) Virtual labs and videos are available from educational sites to reinforce student learning about bacterial growth [9].

References

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