Experimenting with Biofilms

BACKGROUND

Microbiologists have increasingly come to realize that most microorganisms like to live at solid-liquid interfaces. In fact microbial colonization of submerged surfaces is rapid and has important consequences for microbial growth and survival in natural systems. Microbiologists have found that many infections are the result of biofilm formation in the body. This is particularly true in lung infections, and biofilm formation in the lungs is the main cause of death in patients with cystic fibrosis and pneumonia. Biofilms are also the major cause of infections resulting from medical implants. In natural systems biofilms are responsible for the fouling of boat hulls, corrosion of submerged structures, and fouling of pipes. Microorganisms growing in biofilms can also exhibit characteristics not found in suspended cultures. For example, it has been observed on many occasions that growth in biofilms greatly increases levels of microbial resistance to antibiotics. Compounds that inhibit biofilm formation have potential use in medicine, and engineering applications. There is even a new antibicrobial, 'Microban' that is supposed to retard microbial growth on surfaces, and which can be used to impregnate materials such as children's toys or even socks and.

In this exercise we will count the number of cultivatable bacteria per unit area on glass slides that have been submerged in pond water for 3 days. We will treat the pond water with either a nutrient solution, or with chlorine bleach to see its effect on biofilm formation.

MATERIALS

Per group of 2:

- 1 rubber policeman
- 1 P20 pipette or sterile dropper
- 1 nutrient agar plate
- 1 50 ml sterile tube
- 1 tube of 10ml 1xPBS
- 5 tubes of 0.9 ml Phosphate Buffered Saline
  (PBS: 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4 in 1L water)
- 1 ruler

EXPERIMENTAL METHOD

1) Transfer 10 ml sterile PBS to one sterile 50 ml sterile tube label 'Tube 1' and set aside.

2) Recover one slide from the biofilm experiment. There are three different treatments (control, +nutrients [10g/L sugar], and +bleach [0.1ml/L]). Each group should only do one slide from one treatment.

3) Rinse the slide briefly and gently over the beaker water. We want to rinse off any pond water from the slide while not dislodging any bacteria that may be attached to the glass surface.

4) Label the 50 ml tube 'Tube 1'. Place the slide in the 50 ml tube and use the rubber policeman to scrape the biofilm off of both sides of the slide and into the PBS solution. You may need to use a 1 ml pipette tip to rinse the slide with the PBS solution several times to remove all biofilm material.

5) When the biofilm is removed, cap the 50 ml tube and mix aggressively until as much of the biofilm is suspended in solution as possible.

6) Label the 5 tubes of sterile PBS tube 2 through tube 6. Transfer 0.1 ml of the biofilm containing solution from tube 1 (the 50ml tube) to tube 2. Mix the tube well and then continue sequentially in this manner until you have carried the dilution through all 5 tubes.

7) Use a marker to divide 1 nutrient agar plate into 6 equal wedges and label the wedges 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}.

8) Place three evenly spaced spots of 10 ul from tube 1 into the wedge labeled 10^{-3}. Next, place three evenly spaced spots of 10 ul from tube 2 into the wedge labeled 10^{-4} of the NA plate (since 10 ul is 0.01 ml when you place 10 ul of a 10^{-1} dilution onto the plate you have a total dilution of 10^{-3} ml). Next place three evenly spaced spots of 10 ul from the tube 3 dilution into the wedge labeled 10^{-5}. Continue in this fashion until all of the wedges have been used. Be careful not to move the plate until the spots have dried. It may take several hours for the spots to dry completely and to prevent dripping or smearing the plates should be handled carefully and not inverted until the spots are dry.

9) Incubate the plates at room temp for 2-7 days.

10) Count the number of colonies in the most dilute spots where colonies occur. Divide the average number of colonies that you obtain by the dilution factor to get the total number of cultivatable bacteria present on the glass slide. Then divide by the surface area to get the number of cells per cm^2.

ALTERNATIVES

This exercise can be used to test many different hypotheses about the factors that contribute to biofilm formation and methods for reducing biofilm formation. For example, this experiment can be used to compare: biofilm formation in different water sources (tapwater, pond, river, etc) or by different bacterial cultures, the effect of various biocides on biofilm formation (soap, ethanol, bleach, etc), the effect of various surfaces or surface coatings on biofilm formation (latex paint, oil paint, polyurethane, wax, etc), the effects or temperature, mixing, time, etc. You can also allow your students to design and test their own hypotheses.