Lab 1: Caffeine Mutagenesis of *Streptomyces* Protocol (Week 2)

Materials required (per pair of students)

- Bunsen burner
- Frozen spore suspension of *Streptomyces* in 20% Glycerol (2 mL per student)
- Sterile distilled water in 9 ml aliquots in universal bottles (for dilutions) (29 per student [4 series of 7 dilutions + 1 for initial dilution of spores])
- Nutrient agar plates (8 plates per student)
- Disposable plastic spreaders (4 per student)
- Sterile microfuge tubes
- 1 ml pipettes and tips
- 30°C incubator room
- Marker pen to write on microfuge tubes and plates
- 10 mg/mL caffeine solution (1 mL per student)

Protocol 1.1

All steps should be carried out aseptically. Where directed to mix well, close the microfuge tube and invert it 5-6 times to mix.

1. Pipet 0.5 mL of the spore suspension into a microfuge tube. Add 0.5 mL sterile distilled water. Mix well. This is your control (label the tube, "0 CAFF").

   Prepare your experimental (+CAFF) treatments as follows:
   a. Pipet 0.5 mL of the spore suspension into a microfuge tube. Add 0.5 mL 10 mg/mL caffeine. Mix well. Label the tube, "5 mg/mL CAFF".
   b. Pipet 0.5 mL of the spore suspension in to a microfuge tube. Add 0.25 mL 10 mg/mL caffeine and 0.25 mL distilled water. Mix well. Label the tube, "2.5 mg/mL CAFF".
   c. Pipet 0.5 mL of the spore suspension into a microfuge tube. Add 0.125 mL 10 mg/mL caffeine and 0.375 mL distilled water. Mix well. Label the tube, "1.25 mg/mL CAFF".

   Incubate your +CAFF treatments on the benchtop for 10 minutes. While you wait, label your tubes and plates as described for steps 2 and 3. (See the Note on Labelling Technique, p. 7.)

2. Dilute the control and + CAFF spore suspensions using the 9 mL aliquots of sterile distilled water.

   a. To do this, first label your tubes (10^{-1} through 10^{-7} for both the control and + CAFF samples. (You should have 28 tubes in total.))
   b. Take the 1 ml of the spore suspension, and add this to 9 ml of sterile distilled water in the 10^{-1} tube, and mix well.

c. Using a new pipet tip, remove 1 ml from the 10^{-1} tube and add this to 9 mL of sterile distilled water in the 10^{-2} tube, and mix well.
d. Repeat steps b and c until you reach the 10^{-7} dilution.
e. At this point you should have four dilution series (0 CAFF, 1.25 CAFF, 2.5 CAFF, and 5 CAFF, with 7 tubes each) of *Streptomyces* spores ready for plating onto agar plates.

3. Label agar plates with your initials and the date and along with the following:

a. 0 CAFF, 10^{-6}
b. 0 CAFF, 10^{-7}
c. 1.25 mg/mL CAFF, 10^{-6}
d. 1.25 mg/mL CAFF, 10^{-7}
e. 2.5 mg/mL CAFF, 10^{-6}
f. 2.5 mg/mL CAFF, 10^{-7}
g. 5 mg/mL CAFF, 10^{-6}
h. 5 mg/mL CAFF, 10^{-7}

4. Aseptically add 0.1 ml of the corresponding dilution to your labelled agar plate and spread across the surface using a plastic disposable spreader. (Plate the 10^{-7} dilution first, and then use the same spreader to plate the 10^{-6} dilution, to minimize the amount of plastic waste. Use a new spreader for the control and for each of the different + CAFF treatments.)

5. Allow the plates dry for 10 minutes, invert and then place in boxes for incubation at 30°C for 3 days.

Protocol 1.2 - *In the next laboratory session* (Week 4)

1. Remove plates from the incubation boxes and count the plate dilutions that have between 30 and 300 colonies per plate. Do this for all samples.

2. Compare the colonies obtained on the 10^{-7} plates for the untreated plates and those on the equivalent dilutions of +CAFF plates. Can you identify over-producing mutants of the pigmented antibiotic Actinorhodin (Blue) or undecylprodigiosin (Red)?

3. Enter your data in Table 1.1 and upload your data to MyPlace to share with the class.

4. Using the class data, work out the mean CFU survival for each caffeine concentration.
   a. Using these data, plot a caffeine concentration versus survival graph (x-axis = caffeine concentration; y-axis = CFU/ml). Determine which caffeine concentration gives 99% killing.

Table 1.1. Caffeine mutagenesis of *Streptomyces coelicolor* A3(2).

Plate	**Number of colonies (CFU)**	**Observations (note +act or + red colonies or any unusual phenotypes)**
0 CAFF, 10^{-6}		
0 CAFF, 10^{-7}		
1.25 mg/mL CAFF, 10^{-6}		
1.25 mg/mL CAFF, 10^{-7}		
2.5 mg/mL CAFF, 10^{-6}		
2.5 mg/mL CAFF, 10^{-7}		
5 mg/mL CAFF, 10^{-6}		
5 mg/mL CAFF, 10^{-7}		