Experiment 5: Microscopy: *Candida* morphological alterations

Protocol 5.1: Set up cultures for *Candida* microscopy (Week 8)

1. You will be provided with a 10 mL *Candida albicans* culture in PIM medium. You will use this culture to set up 4 cultures with different concentrations of Antibiotic X (0, 10, 100, and 300 μM X). Label 4 sterile universal tubes appropriately.

2. Aseptically transfer 1 mL of culture into the four labelled sterile universal tubes

3. Add 9 mL of fresh PIM medium to each tube.

4. Add the appropriate volumes of a 0.1 M Antibiotic X solution, so that you achieve final concentrations of 10, 100 and 300 μM of X in three of the tubes. Leave the fourth tube without X as a negative control. Let the cultures grow overnight at 37°C.

Table 5.1 Antibiotic X dilutions (prepared using 0.1 M antibiotic X)

Tube	Final [Antibiotic X] (μM)	Amount of X to add
1	0	
2	10	
3	100	
4	300	

Protocol 5.2: *Candida* microscopy (Week 10)

5. **In Week 10**, observe samples of each of your four cultures under the microscope. Were there any morphological alterations? Do you observe any other changes? Could you explain these changes?

6. Use a haemocytometer to count yeast and filamentous cells. Calculate the % of each morphological type. Record your results in Table 5.2.

Table 5.2: Morphological alterations of *Candida albicans* after Antibiotic X treatment.

Antibiotic X concentration	**Number of yeast cells**	**Number of hyphal cells**