**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 37oC.

**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)**

1. **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?
2. 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** |
|  |  |  |
|  |  |  |
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