OD-based growth model

Introduction

Scattering of light by a replicating population over time, and hence the changing OD is a measure to model the growth of a fungal species over time. This experiments aims to determine the growth rate and lag time of fungal species identified in our surroundings.

Materials

- Fungal samples cultures on slant PDA(Potato dextrose agar)
- Potato dextrose broth(PDB)
- Test tubes
- Phosphate buffered saline(PBS)
- Tween 20
- 10mL Falcon tubes
- Vortex machine
- Hemocytometer
- 96-well plate
- Plate reader

Procedure

Preparation of spore suspension

1. Keep the necessary items in the biosafety cabinet and switch on the UV light for sterilization.
2. Switch off the UV light after 15 mins and wipe off the work area with 70% ethanol.
3. Add 5 mL of PBS to the slant test tube, swirl, and pour the PBS into a falcon tube.
4. Add 0.1% (of total volume of PBS added, 5µL, here) Tween 20 to the tube.
5. Vortex suspension for 15 seconds at 2000 rpm for uniform mixing of spores in the PBS.

Counting of spores using a hemocytometer

6. Clean the hemocytometer with 70% ethanol and kim wipes and place its coverslip covering both the chambers.
7. Load the spore suspension in the two chamber.

8. Place the hemocytometer under microscope and view it under 10X and 20X to clearly visualise the spores.

9. Select the box to be considered according to the size of the spores and count them.

10. Calculate the number of spores in the entire 5ml of spore suspension.

Setting up the 96-well plate

11. To keep the number of spores constant in all the wells, dilute the spore suspension using PBS.

12. Prepare dilutions to make suspension of 4*10^4 spores/mL.

13. 3 wells(triplicate) are to be used for each fungal species in a 96-well plate. Fill the wells with 100µL of PDB.

14. Add 100µL of spore suspension to make the final count of spores in the well as 2*10^4 spores/mL.

15. For blank, add 100µL of PDB and 100uL of PBS.

Well1

	1	2	3	4	5	6	7	8	9	10	11	12
A	1A		2A		3A		Blank					
B	1A		2A		3A		Blank					
C	1A		2A		3A		Blank					
D												
E												
F	1B		2B		3B		Blank					
G	1B		2B		3B		Blank					
H	1B		2B		3B		Blank					

Note: For our experiment , we used 3 fungal species and made 2 separate slant PDA cultures(A and B) for them
1: *Aspergillus niger(*1A and 1B)
2: *Aspergillus versicolor(*2A and 2B)
3: *Rhizopus oryzae(*3A and 3B)

16. Keep the plate in a shaker cum incubateor at 28℃.

17. Take the OD measurements at 605nm in a plate reader for a fixed interval of time.. (For our purpose, we took 4 times a day at an interval of 6 hr for 4-5 days.)

18. Record the reading.

IC50 of an Antifungal

Introduction

IC_{50} of an antifungal here would indicate how much of the chemical would be needed to inhibit, in vitro, the given fungal species. (https://en.wikipedia.org/wiki/IC50)
Lower the IC50, better the efficiency of the antifungal.

Materials

- Fungal samples cultures on slant PDA(Potato dextrose agar)
- Potato dextrose broth(PDB)
- Test tubes
- Phosphate buffered saline(PBS)
- Tween 20
- 10mL Falcon tubes
- Nipagin (antifungal)
- 100% ethanol
- Vortex machine
- Hemocytometer
- 96-well plate
- Plate reader

Procedure

Preparation of spore suspension

1. Keep the necessary items in the biosafety cabinet and switch on the UV light for sterilization.
2. Switch off the UV light after 15 mins and wipe off the work area with 70% ethanol.
3. Add 5 mL of PBS to the slant test tube, swirl, and pour the PBS into a falcon tube.
4. Add 0.1% (of total volume of PBS added, 5µL, here) Tween 20 to the tube.
5. Vortex suspension for 15 seconds at 2000 rpm for uniform mixing of spores in the PBS.

Counting of spores using a hemocytometer

6. Clean the hemocytometer with 70% ethanol and kim wipes and place its coverslip covering both the chambers.

7. Load the spore suspension in the two chamber.

8. Place the hemocytometer under microscope and view it under 10X and 20X to clearly visualise the spores.

9. Select the box to be considered according to the size of the spores and count them.

10. Calculate the number of spores in the entire 5ml of spore suspension.

Use of antifungal-Niapagin

11. To make 0.1g/mL concentration of Nipagin, add 0.1 g of Nipagin to 100% ethanol.

12. Dilutions can be made according to the need using 100% ethanol.

Setting up the 96-well plate

13. To keep the number of spores constant in all the wells, dilute the spore suspension using PBS.

14. Prepare dilutions to make suspension of 4*10^4 spores/mL and 8*10^4spores/mL.

15. 3 wells(triplicate) are to be used for each concentration of Nipagin in a 96-well plate. Fill the wells with 100µL of PDB.

16. Add 50µL of spore suspension from spore concentration of 8*10^4spores/mL to make the final count of spores in the well as 2*10^4 spores/mL.

17. Add 50µL of Nipagin to the triplicates.

18. For blank, add 100µL of PDB and 100uL of PBS.

Well1

<table>
<tr><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th></tr>
<tr><td>A</td><td rowspan="3">Conc. 1</td><td rowspan="3">Conc. 2</td><td rowspan="3">Conc. 3</td><td rowspan="3">Conc. 4</td><td rowspan="3">Conc. 5</td><td rowspan="3">Conc. 6</td><td rowspan="3">Conc. 7</td><td rowspan="3">Conc. 8</td><td rowspan="3">Conc. 9</td><td rowspan="3">Conc. 10</td><td rowspan="3">Conc. 11</td><td rowspan="3">Conc. 12</td></tr>
<tr><td>B</td></tr>
<tr><td>C</td></tr>
<tr><td>D</td><td colspan="3">Fungal spores with ethanol</td><td></td><td rowspan="2">Blank</td><td></td><td colspan="3">Fungal spores with ethanol</td><td></td><td rowspan="2">Blank</td><td></td></tr>
<tr><td>E</td><td colspan="3">Only fungal spores</td><td></td><td></td><td colspan="3">Only fungal spores</td><td></td><td></td></tr>
<tr><td>F</td><td rowspan="3">Conc. 1</td><td rowspan="3">Conc. 2</td><td rowspan="3">Conc. 3</td><td rowspan="3">Conc. 4</td><td rowspan="3">Conc. 5</td><td rowspan="3">Conc. 6</td><td rowspan="3">Conc. 7</td><td rowspan="3">Conc. 8</td><td rowspan="3">Conc. 9</td><td rowspan="3">Conc. 10</td><td rowspan="3">Conc. 11</td><td rowspan="3">Conc. 12</td></tr>
<tr><td>G</td></tr>
<tr><td>H</td></tr>
</table>

Row A, B, C: 100µL PDB+50µL spore suspension of 8*10^4 spores/mL (*Aspergillus versicolor*)+ 50µL Nipagin(Conc in µg/mL)

Row E, F, G: 100µL PDB+50µLspore suspension of 8*10^4 spores/mL (*Rhizopus oryzae)*+ 50µL Nipagin(Conc in µg/mL)
D1, D2, D3: 100µL PDB+50µL spore suspension of 8*10^4 spores/mL (*Aspergillus versicolor)*+ 50µL 100% ethanol
D7, D8, D9: 100µL PDB+50µL spore suspension of 8*10^4 spores/mL (*Rhizopus oryzae)*+ 50µL 100% ethanol
E1, E2, E3: 100µL PDB+ 100µL spore suspension of 4*10^4 spores/mL (*Aspergillus versicolor)*
E7, E8, E9: 100µL PDB+ 100µL spore suspension of 4*10^4 spores/mL (*Rhizopus oryzae)*

19. Keep the plate in a shaker cum incubateor at 28℃.

20. Take the OD measurements at 605nm in a plate reader for a fixed interval of time.. (For our purpose, we took 4 times a day at an interval of 6 hr for 4-5 days.)

21. Record the reading.