High-throughput Assay for Screening Fungal Isolates against Polyphenolic Compounds

This protocol is published without a DOI.

Jana M U'Ren[1], Megan Nickerson[1]

[1]University of Arizona

Megan Nickerson
University of Arizona

NOV 16, 2022

ABSTRACT

This protocol was developed for the screening of fungal isolates against polyphenolic compounds to test their capacity for detoxification.

PROTOCOL CITATION

Jana M U'Ren, Megan Nickerson 2022. High-throughput Assay for Screening Fungal Isolates against Polyphenolic Compounds . **protocols.io** https://protocols.io/view/high-throughput-assay-for-screening-fungal-isolate-cjaeuibe

CREATED

Nov 15, 2022

LAST MODIFIED

Nov 16, 2022

PROTOCOL INTEGER ID

72742

Media and Solution Preparation- Aspergillus nidulans Defined Media

1 Combine:
- 900mL nanopure water
-50mL 20X Sodium nitrate salts
-1mL Trace elements

2 Bring the pH to 6.5.

3 Add 2 grams of Carageenan Type II (0.2% final concentration)

4 Autoclave for 30 minutes on liquid cycle and allow to cool to room temperature. Store at 4C.

Media and Solution Preparation- Glucose solution

5 In a 15 mL conical tube, add 3.2g of glucose.

6 Add enough nanopure water to bring volume to 10 mL and vortex to mix until completely dissolved.

7 In a biosafety cabinet, filter sterilize and make 1 mL aliquots.

Media and Solution Preparation- Polyphenol solutions (excluding Var

8 Prepare 3 5mL tubes with 3 concentrations of each polyphenol dissolved in **autoclaved nanopure water**.
Note: Concentrations (when diluted in wells): 2000ug/mL, 1000ug/mL, and 500ug/mL. **Actual concentrations of stock: 40,000 ug/mL,20,000 ug/mL, and 10,000 ug/mL**

9 For inoculating two plates, dissolve **0.08g**, **0.04g, and 0.02g** of each polyphenol in 2mL of **autoclaved nanopure water**. Vortex thoroughly to mix until completely dissolved.

10 In a biosafety cabinet, using the 0.2um disc filters, filter sterilize 1mL of each solution into a sterile 1.5mL tube.

Media Solution Preparation- Vanillin

11 Due to issues with solubility, the amount of vanillin used has to be scaled down. For inoculating two plates, dissolve **0.02g, 0.01g, and 0.005g** of vanillin in 2mL of **autoclaved nanopure water**. Vortex thoroughly to mix until completely dissolved.

12 In a biosafety cabinet, using the 0.2um disc filters, filter sterilize 1mL of each solution into a sterile 1.5mL tube.

Media and Solution Preparation- Lignin

13 Due to issues with solubility, the amount of lignin used has to be scaled down. For inoculating two plates, dissolve **0.02g, 0.01g, and 0.005g** in 20mL nanopure water in glass jars.

14 Autoclave on Liquid30 cycle.

15 In a biosafety cabinet, aliquot into 2mL tubes.

Inoculate fungal cultures on cellophane (to be done ahead of time)

16 Inoculate ___ 60mm MEA plates +cellophane with ___ isolates and incubate at room temperature until growth is sufficient to proceed. List isolates in your notebook.
- Example: 24 plates with 4 isolates (6 plates per isolate)
- Allow to grow until sufficient mycelium for inoculation (e.g., 1 week)

Prepare liquid fungal inoculum for spectrophotometer (all steps done

17 Using a serological pipette add 20mL of A. nidulans defined carrageenan media to blender cup (20 mL is the minimum amount needed for blender cup to work).

18 Cut mycelium closely around the original inoculum to remove the agar square from the center of the cellophane. Carefully scrape the mycelium from the cellophane, without cutting into the cellophane, and gather into a ball using the broad scalpel. Pick up the mycelium ball using the fine forceps and place in blender cup.

19 Blend four times at one-second pulses. Check fragment sizes to ensure sufficient blending.

20 OR
Once isolate has colonized entire plate, pipette 1-5mL of sterile water onto plate. Use sterilized rubber policeman to gently scrape the hyphae off the surface of the media. Using a wide-bore pipette tip, collect the liquid and place a sterile blender cup.

21 Using serological pipette, add 0.2% carrageenan type II media until final volume is 20mL in blender cup. (e.g., 20mL final volume - ___mL collected from plate = _____mL 0.2% carrageenan media to add.)

22 Blend four times at one-second pulses.

23 Transfer 1000uL of blended culture (while pipette mixing) with a 5mL pipette to three spectrophotometer cuvette replicates.

24 Transfer 1000uL of A. nidulans defined carrageenan media from 5mL tube into cuvette (this will be the blank cuvette).

Read samples in spectrophotometer (at 600nm)

25 Place blank cuvette with just 1000uL of A. nidulans defined carrageenan media into reading position (with the cuvette side with the arrow closest to the screen) and press the 0A/100%T button to blank the sample.

26 Place sample in reading position and press the “arrow passing through the cuvette” button. Record absorbance.

27 A final reading of **>0.44** is needed to reach the appropriate hyphal fragment density. If the absorbance of your sample is lower than this, harvest more hyphae from plates and repeat blending procedure in a new blender

cup.

Dilute blended culture

28 When a reading is **>0.44** you need to dilute the blended inoculum with A. nidulans defined carrageenan media to yield **4 ml** of culture inoculum with an absorbance of 0.22. Use a 5 mL conical tube for the dilution.
- Example: If a sample has an absorbance of 0.50, add 1.32mL of blended culture from blender cup to 1.68mL of 0.2% carrageenan type II media where 0.5(X)= 0.22(3mL) so X=1.32 ml

Inoculate 96-well Plates (All steps to be performed in biosafety cabi

29 Once dissolved, add **5uL of sterilized phenol solution** to the respective wells.

30 Add **5uL of autoclaved nanopure water** to controls (colored purple; media without phenols but with fungi).

31 Combine **1.8mL of blended culture at absorbance 0.44 and 12.5mL** of A. nidulans defined carrageenan media. Mix well by inversion to evenly distribute the hyphal fragments in the media.

32 Add 0.89 mL volume glucose solution to a final concentration of 2% glucose. Immediately proceed to next step so that hyphae don't settle in the media.

33 Inoculate 95uL of GLUCOSE CONTAINING solution for wells corresponding to the proper isolate +glucose. Also inoculate the corresponding **positive control wells**. **(do not inoculate wells for the media only controls in Row A)**.
- Do NOT push pipette plunger past the last stop because this will create bubbles, which will cause readings to be inaccurate. If bubbles form try to pop with a sterile pipette tip.

34 Place lid on the plate and parafilm for transport. Immediately measure the plate at 595, 660, and 750nm (cell density only) on plate reader.

35 After reading, re-parafilm plate and place in plastic bag with wet paper towels to keep plates from drying out. Be careful to keep the plate upright at all times.

36 Store plates in an aluminum foil lined container to keep out the light.

37 Read plates every 3 days for a total of 24 days (tentative, depends on isolate growth).

38 After 24 days, record visual observations and take picture of plates

Inoculate 96-well Plates WITHOUT Glucose (All steps to be performe

39 Once dissolved, add **5uL of sterilized phenol solution** to the respective wells.

40 Add **5uL of autoclaved nanopure water** to controls (colored purple; media without phenols but with fungi).

41 Combine **2.0mL of blended culture at absorbance 0.44 and 14.0mL** of A. nidulans defined carrageenan media. Mix well by inversion to evenly distribute the hyphal fragments in the media.

42 Immediately proceed to next step so that hyphae don't settle in the media.

43 Inoculate 95uL solution for wells corresponding to the proper isolate +no glucose. Also inoculate the corresponding **positive control wells**. **(do not inoculate wells for the media only controls in Row A)**.
- Do NOT push pipette plunger past the last stop because this will create bubbles, which will cause readings to be inaccurate. If bubbles form try to pop with a sterile pipette tip.

44 Place lid on the plate and parafilm for transport. Immediately measure the plate at 595, 660, and 750nm (cell density only) on plate reader.