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Malassezia culture on modified Dixon media

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Hao Li[1], Genevieve Ong[1], Shirlyn Goh[1]

[1]IBN, A*STAR

Hao Li Research Group

Amy Hao Li
National University of Singapore, Agency for Science, Techno...

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Protocol status: Working
We use this protocol and it's working

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Abstract

The protocol describes the procedures to culture Malassezia species in modified Dixon (mDixon) media. It includes the recipe for mDixon, how to start the culture from freezer stock, culture maintenance and how to make frozen stocks.

Materials

Reagents:

- Trypan blue
- modified Dixon broth pH 6 (Refer to the recipe given)

Materials

- Weighing boat
- 1.5 mL Eppendorf tubes
- Parafilm
- Serological pipettes
- Pipette gun
- Inoculation loops
- Microscopy glass slides and coverslips, or haemocytometer
- Corning® 125 mL Polycarbonate Erlenmeyer Flask with Vent Cap
- p20 micropipette and micropipette tips
- p200 micropipette and micropipette tips
- p1000 micropipette and micropipette tips

Equipment

- Microscope
- Incubator shaker with suitable size clamps
- Weighing balance
- pH meter

Safety warnings

Dispose all wastes into the appropriate disposal bins.

- Dispose of all weighing boats and micropipette tips into the chemical waste bin
- Dispose of all biohazardous materials (e.g. inoculation loop) into the biohazard bin
- Dispose of all serological pipettes into the sharps bin or biohazard bin when involved in the handling of biohazardous materials
- Dispose of all glass slides/haemocytometers used in the sharps bin

Preparation of modified Dixon Broth

1 Place a magnetic stir bar into an autoclaved beaker with Mili-Q water and turn on the magnetic stirrer.
- Set the temperature at 60-70ºC
- Temperature and stirring speed can be adjusted accordingly while preparing the media.

2 Add the following ingredients while stirring.
- Add solid ingredients first before adding liquid ingredients.

	Ingredient	Amount needed for 1L
	Peptone	6 g
	Desiccated Oxbile (Bovine Bile)	20 g
	Malt Extract	36 g
	Oleic Acid	2 mL
	Tween 40	10 mL
	Glycerol	2 mL

Modified Dixon Broth Ingredients

Note: Tween 40 takes a long time to dissolve completely, so it's recommended to prepare a working stock of 10% Tween 40 beforehand and add 100 mL of that. The same can be done for glycerol.

Bacto Agar can be added to 1.5% (w/v) at the last step (after pH and volume adjustment) to make mDixon agar plates.

3 Measure the pH of the media using a pH Meter and adjust accordingly using hydrochloride acid or sodium hydroxide to pH 6 .

Starting Culture from Frozen Stock

4 Retrieve the frozen stock from -80˚C and scrap some of the frozen stock using a sterilized pipet tip. If possible, this step should be performed in a Biosafety Cabinet to minimize contamination.

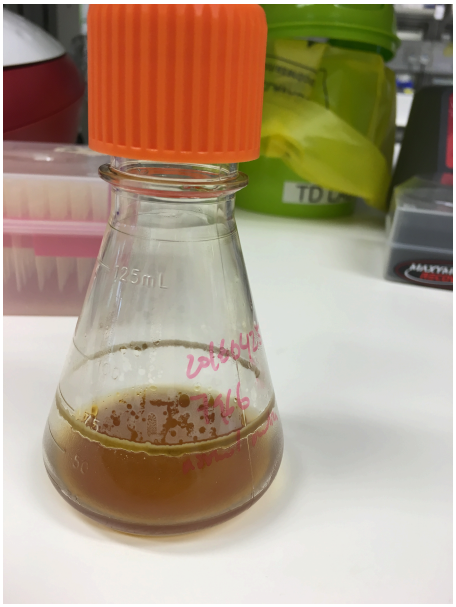
5 Inoculate 12 mL of pre-warmed mDixon culture in a 125 mL flask with the frozen stock. Alternatively, the frozen stock can be streaked out onto a mDixon agar plate.

6 Incubate culture at 32˚C. For planktonic culture, shake flask at 150 rpm. For fast growing *Malassezi*a species (eg. *M. furfur*), planktonic culture will be confluent in 2 days after inoculation. For slower growing species (eg. *M. globosa*), planktonic culture will be confluent in 3-4 days after inoculation.

Maintaining Planktonic Culture

7 Warm up the media in a 37˚C water bath.

8 Retrieve the culture flask and aspirate most of the culture, leaving around 1 mL of culture.
- Bleach the aspirated culture for at least 20 min before disposing.



M. globosa culture in mDixon. Note that *Malassezia* cultures typically have two phases: a liquid planktonic phase and a solid sessile phase that appears as a ring around the air-liquid interface.

9 Pipette in 12mL of the warmed media into the shaker flask and place the shaker flask back into the incubator shaker.

Monitoring Viability

10 Add 10 µL of trypan blue stain onto a glass microscopy slide and mix with 10 uL of the fungal culture.

11 Use a light microscope with at least 40X lens to check fungal growth. Most of the fungal cells would be stained light blue, while the dead cells are stained dark blue.
Note: cell viability and count can also be done at this point using a hemacytometer.

Freezing Down Pellet

12 Grow planktonic culture to late log/early stationary phase.

13 Spin culture down at 8000 rpm for 5 mins and aspirate the supernatant.

14 Resuspend culture pellet in the same volume of 25% glycerol in mDixon, aliquot in screw cap freezer tubes and freeze at -80˚C.