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Malassezia culture on modified Dixon media

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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

Troubleshooting



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 60-70°C
 - 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.

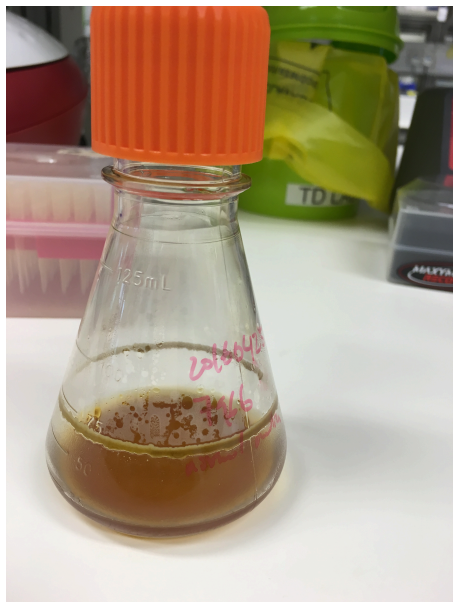
- 3 Measure the pH of the media using a pH Meter and adjust accordingly using hydrochloride acid or sodium hydroxide to  6 .

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 *Malassezia* 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 μ L of trypan blue stain onto a glass microscopy slide and mix with 10 μ L 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 .