

Mar 04,
2020

Ectoplasmic Net (EN) formation in *Aurantiochytrium limacinum* (ATCC MYA-1381)

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1 Works for me dx.doi.org/10.17504/protocols.io.bc7hizj6

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ABSTRACT

Protocol for ectoplasmic net (EN) formation in *Aurantiochytrium limacinum* (ATCC MYA-1381).

BEFORE STARTING

Grow cells in GPY media for 3 days

d-GPY plating

- Take 150 µl of 3 day old cells grown on GPY media and plate it on d-GPY plates.
 - The recipe for d-GPY media is as follows,

Components	Quantity (%)
Yeast extract	0.05
Peptone	0.1
Glucose	0.2
Prepare 1/2 saline media by adding appropriate amount of instant ocean and water.	
For agar plates	1.5% agar

- Incubate the plates at 25 °C Overnight



Add appropriate antibiotics to minimize bacterial contamination.

Zoospore production

- After overnight incubation (~24 h), add just enough artificial sea water ((with appropriate antibiotics) to cover the entire plate.
 - Incubate the plates for 03:00:00 25 °C

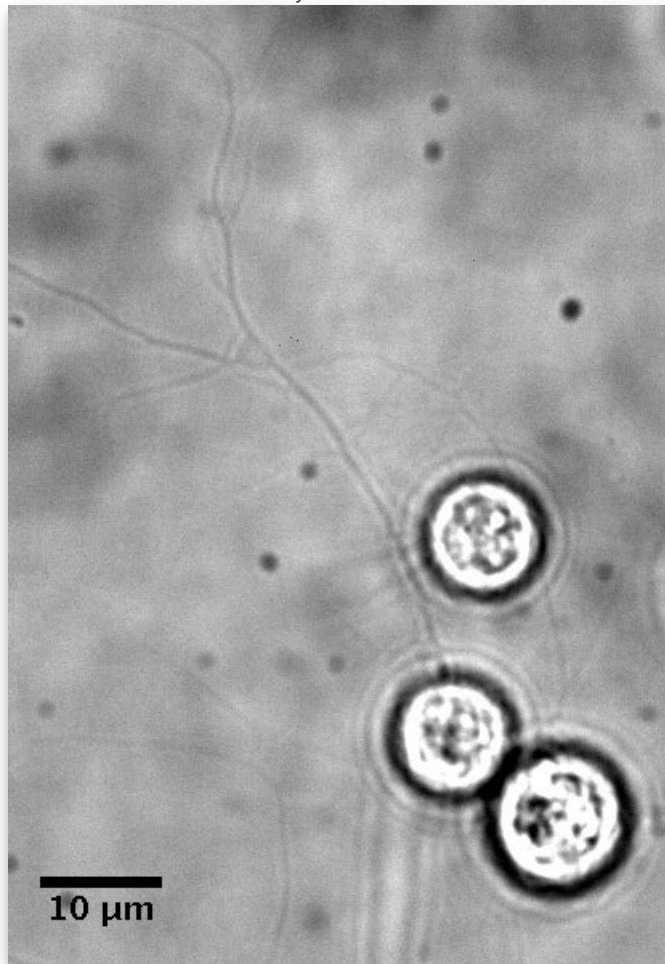


Do not disturb the plates!

- After the incubation, take a small aliquot from the sea water layer and observe for the presence of zoospore.
- The zoospore production starts in about 90 mins, but it peaks around 3 h.

Settlement and EN formation

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 - Take an empty sterile petridish and place a sterile microscopic slide.
 - Add just enough A1 media (with appropriate antibiotics) on to the petridish so that the microscopic slide is immersed.
 - Alternatively, a cover slip can also be used.
 - Aspirate around **50 µl** to **100 µl** of zoospores without disturbing the plate.
 - Carefully drop the zoospores all over the microscopic slide.
 - Cover the petridish and incubate at **25 °C** **Overnight**
 - Observe the EN the next day.



EN starts forming after 90 mins incubation in the slides. If the experiment demands to observe EN formation, the zoospores can be allowed to settle on a glass bottom petridish for overnight timelapse microscopy.



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