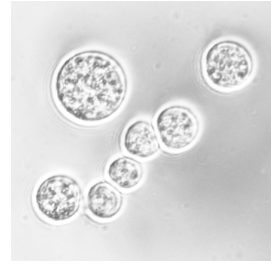


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Synthetic media (A1) for *Aurantiochytrium limacinum* (ATCC MYA-1381)

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Protocol status: Working

We use this protocol and it's working

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Abstract

Synthetic media recipe for *Aurantiochytrium limacinum* (ATCC MYA-1381). This media may not be suitable for *Aplanochytrium* and *Oblongichytrium*.

Guidelines

Work under sterile conditions (use flame). Use gloves, wipe the bench with 70% ethanol.

Safety warnings

- ! The autoclaved bottles and solutions will be hot! Always wear gloves and appropriate clothing before handling autoclaved materials.
Heavy metals are toxic for the environment and need to be discarded accordingly.

Before start


Thaw the antibiotics on ice.

Autoclave empty media bottles for trace metal mix and vitamins.

Autoclaved water for preparing 1X stocks.



5x A1stock

- 1 Measure and add the following components to an appropriate media bottle. Make up the solution to desired volume with water. Autoclave for  00:20:00 at 15 PSI and

 121 °C

Compo nents	g/L
CaCl ₂ ·2 H ₂ O	2.5
Glycerol	150
KCl	2
KH ₂ PO 4	20
maleic acid	29
MgCl ₂	4
Na ₂ SO 4	15
NaCl	72
Sodium - glutama te hydrate	125
Tris base	30

Note

The composition is for **5X**

Trace metal mix

- 2 Measure and add the following components to an amber bottle. Make up the solution to desired volume with water. **Filter sterilize** and refrigerate the trace metal mix.

Compo nents	g/L



FeSO ₄ · 7H ₂ O	50
CuSO ₄ · 5H ₂ O	3.9
ZnSO ₄ · 7H ₂ O	4.4
MnSO ₄ · H ₂ O	1.5
Na ₂ MoO ₄ · 2H ₂ O	0.1
CoCl ₂ · 6H ₂ O in 0.6 M HCl	0.2

Note

Do not autoclave!

Vitamins

- 3 Measure and add the following components to individual amber bottles. Make up the solution to desired volume with water. **Filter sterilize** the vitamins separately and refrigerate.

Components	g/L
Thiamine·HCl (B1)	5.00E-05
Cyanocobalamin (B12)	5.00E-06

Note

Do not autoclave!

Prepare working stock (1X)

- 4 For  1 L ,
Add the stock solutions to an autoclaved bottle.

Stock solution	Volume (mL)
A1 stock (5X)	200
Vitamin B1 (1000X)	1
Vitamin B12 (10000X)	0.1
Trace metal mix (400X)	2.5



Make up the media to  1 L using autoclaved Millipore water.

Note

To prevent bacterial contamination, add  1 mL of 100 mg/mL Ampicillin to the final media.

One of the vitamins may not be added for the A1 vitamin drop out media.

A1 plates

- 5
 - Prepare 1X stock of A1 media (do not add trace metal and vitamins).
 - Add 1% (w/v) agar in to the 1X A1 media.
 - Autoclave for  00:20:00 at 15 PSI and  121 °C . Allow the solution cool down.
 - Add the trace metal mix, vitamins and appropriate antibiotics.
 - Pour the plates and store it in the refrigerator until use.