BY-2 Cell culture maintenance

Project: iGEM KUL 2021 lab notes
Authors: Sarah Vorsselmans
Entry Created On: 2021-07-20 03:39:33 PM +0000
Entry Last Modified: 2021-10-20 09:41:05 AM +0000
Export Generated On: 2021-10-20 06:28:06 PM +0000

THURSDAY, 7/1/2021

Keywords:

BY-2, liquid cell suspension culture, contamination control, growth optimization

Background:

BY-2 cells are a plant cell line established from *Nicotiana tabacum* cultivar Bright Yellow - 2 of a tobacco plant. They have a relatively high homogeneity and high growth rate. Transformation with *Agrobacteria tumefaciens* is more efficient in this cell line. The cells do cluster pretty easily in liquid cell suspension culture.

Rationale:

In this experiment overview we'll describe how we maintained the BY-2 liquid cell suspension culture, minimized contamination and tried to optimize growing conditions in our lab.

Key protocols:

BY-2 Transformation Protocol

Relevant parts of the protocol also copied below

Materials:

- **BY-2 vitamine stock** (*50 mL H_20)*
  - 2,4D (auxin, dissolve in 1 mL ethanol) **0.02 g**
  - Thiamine **0.05 g**
  - Myo-inositol **5 g**
  - *Filter sterilize, aliquot 10 mL in 1 mL falcon tubes, store at –20° C*
  - *Prior to use, melt and vortex to redissolve*
- **Plant medium** *for BY-2 liquid cultures (1 L)*
  - MS salts (no additives Duchefa) **4.302 g**
  - KH_2PO_4 **0.2 g**
  - Sucrose **30 g**
  - pH with **1 M KOH 5.8**
  - *Autoclave*
  - *Prior to use, add 1 mL of BY-2 vitamin stock*
- **Plant medium** *for BY-2 solid cultures (1 L)*
  - MS salts (no additives Duchefa) **4.302 g**
  - KH_2PO_4 **0.2 g**
  - Sucrose **30 g**
  - Phytagel™ **6.5 g**
  - pH with **1 M KOH 5.8**
  - *Autoclave*
  - *Prior to use, add 1 mL of BY-2 vitamin stock*

- **Erlenmeyers** (250 mL)
  - red screw caps / metal cap (which allows air flow)
  - surgical tape

Protocol: Growing the cultures (dilute 7-day BY-2 cell suspension culture)

1. Work in sterile plant flow hood.
2. Add **40 mL** of medium to each erlenmeyer.
3. Inoculate with **1 mL** of the 7-day BY-2 liquid culture (the volume **depends** a bit on the history of the cells, after a while, they grow faster) the cells using a **5/10 mL** sterile Pasteur pipette so you don't damage the cells. Shake the erlenmeyer before use since the cells sink fast.
4. Release the cells directly into the medium.
5. Unscrew the cap half/one turn, and seal with surgical tape (permits airflow).
6. Incubate in a DARK shaker, **28°C, 150 rpm.**
7. Refresh each week, same time, same procedure, normally **1/40** dilution of a 7-day old culture.
8. Cells can be kept viable to a week when stored at **4°C.**
9. To maintain a backup culture, grow the 4 mL of cells on a solid plate. As such it can be grown at **25°C** for up to one month. Refresh when browning of the callus starts to appear.

Experimental details:

THURSDAY, 7/15/2021

Went to prof. Filip Rolland's lab to check if all the materials are ready. That way the BY-2 cells can be grown in the right environment immediately after arrival in Leuven.

FRIDAY, 7/16/2021

Ghent (Jacobs Lab - VIB-UGent Center for Plants Systems Biology)

BY-2 training in growing (& transforming - see BY-2 Transformation work)

--> BY-2 dilution (see "growing BY-2" in protocol linked above)

We also took two WT BY-2 cell lines with us (transported in 50 mL falcon tubes) @ RT. One diluted on Thursday 15/7/2021 (1-day old culture - line A) and one on Thursday 8/7/2021 (8-day old culture - line B).

Leuven (Filip Rolland lab - Moleculaire Biotechnologie van Planten en Micro-organismen)

- 1-day old BY-2 culture (line A)
  - Transfered from 50 mL falcon tube to sterile erlenmeyer with metal cap (+ surgical tape)
  - Placed in shaking incubator @ 28°C
- 7-day old culture (line B)
  - Placed @ 4°C

MONDAY, 7/19/2021

Check up on BY-2 cells

- BY-2 culture in incubator (line A) growing well
- Other BY-2 culture (line B) still @ 4°C

TUESDAY, 7/20/2021

Plant media and vitamins were made according to BY-2 protocol from prof. Thomas Jacobs' lab: BY-2 Transformation Protocol + protocol described above

Plant medium notes:

- MS salts with additives (thiamine, myo-inisitol and other vitamins) was used instead of MS salts without additives

BY-2 vitamin notes:

- The vitamins were then sterile filtered using a syringe and a syringe filter. They were aliquoted into 10ml aliquots and stored at -27°C for further use

THURSDAY, 7/22/2021

BY-2 culture maintenance

- Vitamins added to the plant medium (1 mL/L)
- Line A and B were diluted 1/40 according to the protocol BY-2 Transformation Protocol
- The metal lid on the erlenmeyer was not closed all the way (to allow air circulation). Surgical tape was applied around the lid and placed in an incubator at 28°C.
- Line B has turned grayish. It might not be optimal for us to keep it refridgerated (@ 4°C) for 7 days

BY-2 calli backup

Making BY-2 backup of WT calli on plates

- 0.65 g of plant agar was added to 100 mL of MS media & autoclaved

FRIDAY, 7/23/2021

Pouring plates with plant agar --> does not solidify --> no BY-2 backup calli made yet

MONDAY, 7/26/2021

BY-2 cell maintenance

- Line A looks good
- Line B has mold --> removed from incubator

--> Make new BY-2 media to be sure contamination does not spread.

WEDNESDAY, 7/28/2021

New BY-2 media made

THURSDAY, 7/29/2021

- Line A diluted 1/40 into A1 and A2
- Keep original A @ 4°C for 7 days as backup

FRIDAY, 7/30/2021

Pouring plates with plant agar --> still does not solidify well enough --> no BY-2 backup calli made yet

MONDAY, 8/2/2021

Pouring plates

Use Phytagel™ instead of plant agar. This does solidifies very fast and does not liquify again by microwave.

2 different concentrations of Phytagel™ used:

- 0.65 g Phytagel™ in 100 mL of MS-media (x2) --> this one works best
- 0.25 g Phytagel™ in 100 mL of MS-media (x2)

4 mL of BY-2 cells from line A1 (x2) & line A2 (x2) used to plate each

WEDNESDAY, 8/4/2021

BY-2 cultures already look thick

THURSDAY, 8/5/2021

Add 4 mL of MS media to liquid cultures to stretch the growth to 8 days. This fits our transformation protocol better.

FRIDAY, 8/6/2021

- Line A1 has mold growing in it -> trashed
- Line A2 diluted 1/40
- Keep 8-day A2 @ 4°C as backup

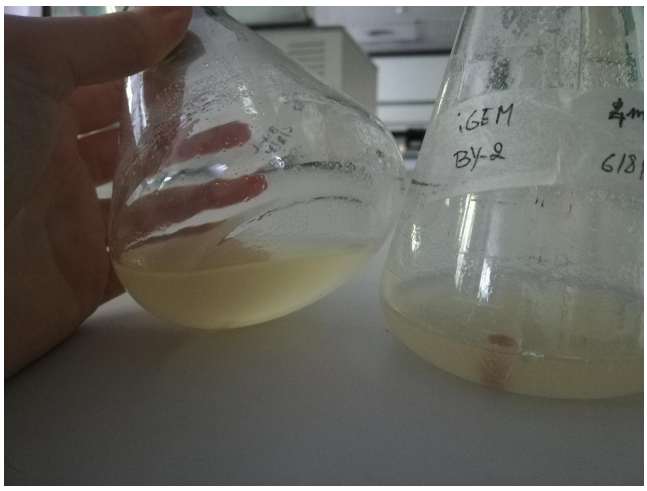
MONDAY, 8/9/2021

MOLD :(

- [ ] The 4 mL culture contains a single round orange fungal 'blob', about 1.5 cm in diameter
- [ ] The 3 mL culture contains 2 smaller orange specs
- [ ] The 2 mL seems clear but we think it's just a matter of time
- [ ] The agar plates were poured and cells were added on August 3rd, there are multiple big grey fungal spots visible as well.

--> Update #wet-lab and contact prof. Thomas Jacobs and prof. Filip Rolland

IMG_20210809_104221.jpg



TUESDAY, 8/10/2021

New BY-2 pick up tomorrow from (Jacobs Lab - VIB-UGent Center for Plants Systems Biology)

WEDNESDAY, 8/11/2021

Decontamination effords:

- [ ] Pick up of new BY-2 cells @ Ghent
  - [ ] transported in 3 small glass erlenmeyers
  - [ ] 1 with cap closed (= line A)

- 2 with cap loose + surgical tape (= line B & C)
- Placed shaking @ 28°C

- All media thrown out
- Incubator and hood sprayed with desinfectant and cleaned
- All erlenmeyers cleaned
- Agar plates thrown out
- Antibiotics (vancomycin & cerbenicellin) and MS arrived last Thursday

FRIDAY, 8/13/2021

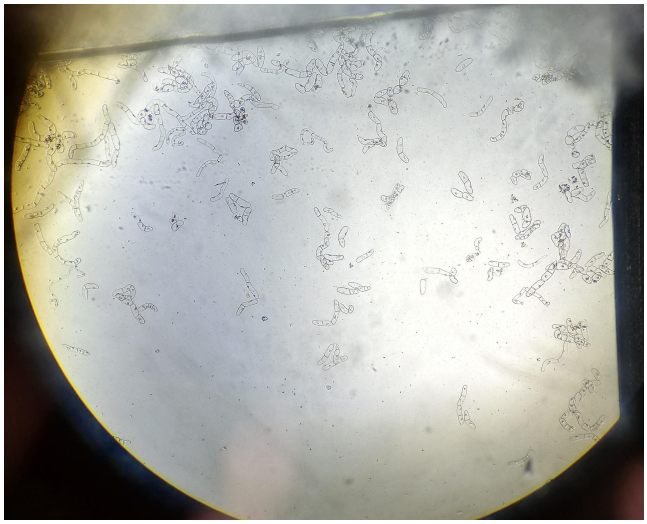
- Make new MS media (with new MS salts; no additives)
- Put 100 mL of MS media in bottle --> will add agar and be autoclaved on Monday (0.65 g/100 mL)
- Filter sterilise vitamins again & store in 1 mL aliquots
- Autoclave erlenmeyers
- Autoclave tips again
- Check on new BY-2 cells -> look good
- Agar plates WT BY-2 mold (2/8/2021) thrown out

TUESDAY, 8/17/2021

Hemocytometer was used to try and count BY-2 cells. This turned out to be impossible since they cluster together and don't spread out evenly enough to count. They would move to the edge of the counter, out of the grid.

Check up on plant cells under microscope

IMG_20210817_151631.jpg



WEDNESDAY, 8/18/2021

The three erlenmeyers with new BY-2 cells are diluted for the first time.

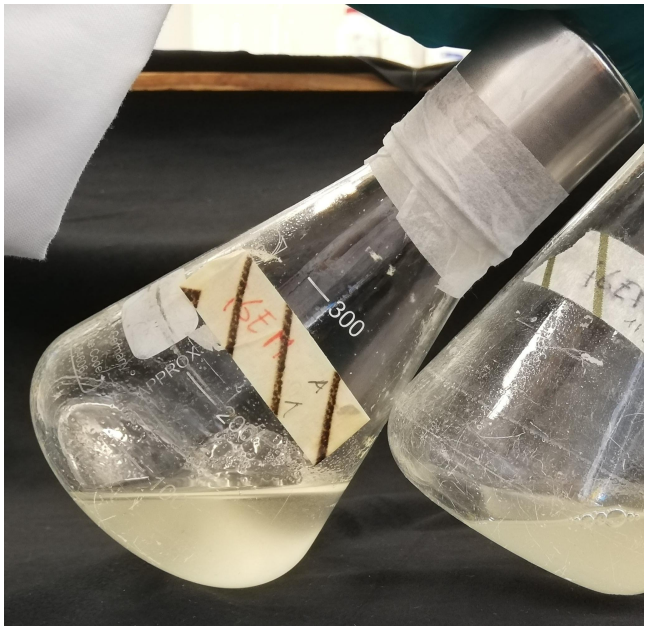
- Line A --> line A1 & A2
- Line B --> line B1 & B2
- Line C --> line C1 & C2

Lines 1 and 2 will be diluted with media from bottle #1 and #2 respecitively to lower the rist of contamination of all lines further.

An extra erlenmeyer only containing media #1 was also placed in the incubator to test for possible contamination from the environment.

The slits in the metal caps itself are now also taped with surgical tape to avoid contamination further.

IMG_20210903_101659.jpg



TUESDAY, 8/24/2021

- [ ] New 1 L of media (divided between 2 bottles --> media #1 and #2)
- [ ] Check on liquid cells
  - [ ] seems that line A (screw cap closed during transport) is much more concentrated than line B and C

WEDNESDAY, 8/25/2021

- [ ] Dilute cells
  - [ ] 1/40 line A 1 & 2
  - [ ] 1/40 line B 1 & 2
  - [ ] 1/40 line C 1 & 2
- [ ] Contamination test
  - [ ] Blank erlenmeyer still clean
- [ ] BY-2 Liquid suspencion cell culture test to preserve space
  - [ ] Growing BY-2 cell suspension cultures in 50 mL falcon tubes from line A1
  - [ ] 4x 50 mL tubes (15 mL MS media and 0.5 mL cells)

FRIDAY, 8/27/2021

BY-2 Liquid suspencion cell culture test to preserve space
--> 50 mL falcon tubes do not work; all cells cluster at the bottom and don't shake

TUESDAY, 8/31/2021

New MS media made

WEDNESDAY, 9/1/2021

Line A (A1 & A2) diluted

THURSDAY, 9/2/2021

Line B & C diluted

--> This was from a 8-day old culture. This is to spread the cell lines over the week to avoid contamination further and give us more options to plan transformation experiments.

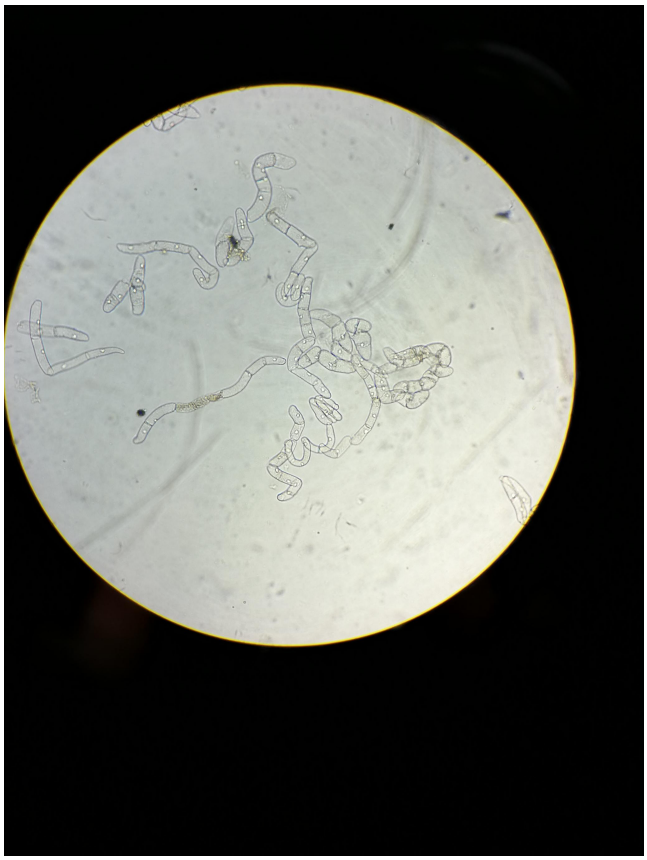
WEDNESDAY, 9/8/2021

- [ ] New MS media made
- [ ] Dilute line A1 & A2

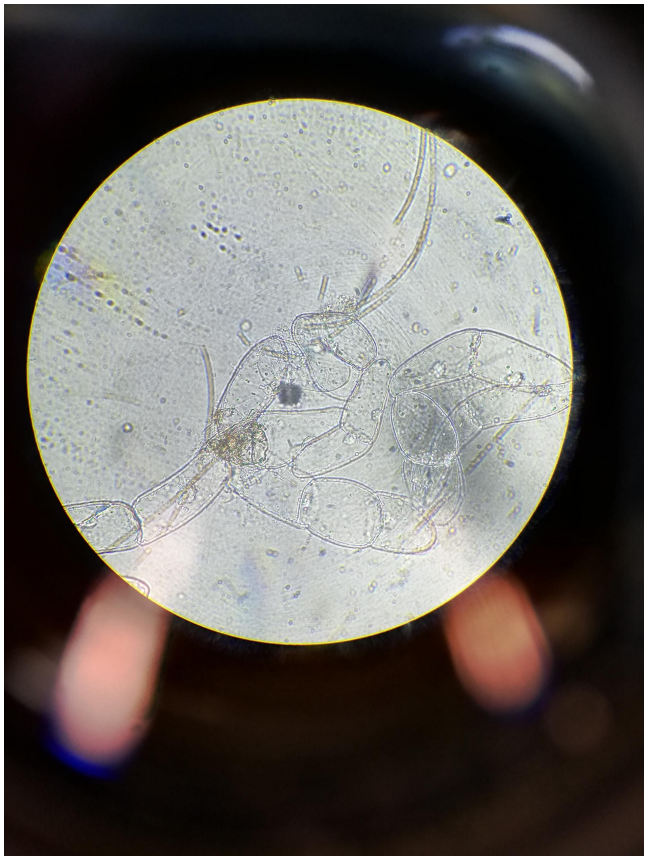
FRIDAY, 9/10/2021

- [ ] Dilute line B, C
- [ ] Look at transformed BY-2 cells under microscope (from deep dish)

IMG_20210910_113905.jpg



IMG_20210910_114045.jpg



TUESDAY, 9/14/2021

New media + agar made

WEDNESDAY, 9/15/2021

Dilute line A

THURSDAY, 9/16/2021

New MS media made

FRIDAY, 9/17/2021

Dilute line B & C

- [ ] Line B2 looked less concentrated than B1, C1, C2

MONDAY, 9/20/2021

Dilute line A

--> This was from a 5-day old culture. This is to spread the cell lines further over the week to avoid contamination further and give us more options to plan transformation experiments.

FRIDAY, 9/24/2021

Line B & C diluted

MONDAY, 9/27/2021

Dilute cell line A

FRIDAY, 10/1/2021

Dilute line B & C
! These cell lines both looked weird: very clumped, weird color and dried up cell ring on erlenmeyer. Maybe something's off with the incubator?

MONDAY, 10/4/2021

Dilute cell line A

MONDAY, 10/11/2021

We threw out all liquid culture cell lines. The squiky incubator (@ 28°C) has been turned off.

Results & conclusions:

- [ ] BY-2 cell suspension culture maintained until the end of the project
- [ ] BY-2 cell suspension culture contamination
  --> Multiple contamination limiting steps were taken -> no new contamination presented itself after that
  - [ ] Throw out all media & wash and sterilize everything again
  - [ ] More possible contamination spots sealed off
  - [ ] More careful handling of cells
  - [ ] More cell lines
  - [ ] Cell line dilutions spread over multiple days
- [ ] Optimize growing conditions
  - [ ] Erlenmeyers setup with metal cap worked best
  - [ ] Growing in 50 mL falcon tubes does not work. We attempted this to save space in the incubator.
  - [ ] Stretching growth 1/2 days to space out cultures worked well.
  - [ ] Storage @ 4°C does not work

Further experiments:

- [ ] Bigger incubator
- [ ] Smaller erlenmeyers with screw cap