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Title: A Simplified Method for *Agrobacterium*-Mediated Transformation of *Phytophthora palmivora*

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

✓ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **02/16/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 15

Number of Shots: 40

Introduction

INTRODUCTION:

REQUIRED: ~~Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Goutom Goswami:** This protocol offers a simple, effective, and reproducible system to transform *Phytophthora palmivora*, and *Phytophthora capsici*.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.4. **Goutom Goswami:** Our protocol allows researchers to study gene functions, determine protein cellular localization, and track pathogen infection dynamics.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.5. **Goutom Goswami:** This protocol may generate a low percentage of false positives and hence requires additional approaches to confirm the true transformants.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.6. **Goutom Goswami:** Application of this transformation system can be extended to other oomycetes and fungi. We will also try to further simplify this protocol in the future.

- 1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. Preparation of *Agrobacterium tumefaciens*

Demonstrator: Goutom Goswami

2.1. To begin, take a new LB agar plate supplemented with antibiotics [1]. Add 20 microliters of LB liquid media to the center of the plate [2] and scoop *Agrobacterium* cells from a previously grown plate with a sterile 1.5-milliliter microcentrifuge tube [3]. Using the bottom and flat base of the tube, spread the cells evenly across the plate [4]. Incubate the plate overnight at 28 degrees Celsius for approximately 16 to 18 hours [5].

2.1.1. Talent placing an agar plate in the hood.

2.1.2. Talent pipetting 20 microliters of LB liquid media onto the center of the agar plate.

2.1.3. Talent scooping *Agrobacterium* cells.

2.1.4. Talent using the flat end of a sterile 1.5 milliliter microcentrifuge tube to spread *Agrobacterium* cells onto an agar plate.

2.1.5. Talent placing the plate into a 28 degrees Celsius incubator.

2.2. Next, scoop the overnight-grown *Agrobacterium* cells using a 1 milliliter pipette tip [1]. Resuspend the cells in 1 milliliter of *Agrobacterium* induction medium by pipetting up and down [2]. Now, transfer the resuspended cells into a sterile 50-milliliter tube covered with foil [3] and add *Agrobacterium* induction medium [4]. Measure the optical density of the sample at 600 nanometers [5] and dilute it with *Agrobacterium* induction medium to achieve an optical density of 0.4 [6].

2.2.1. Talent scooping bacterial cells using a 1 milliliter pipette tip.

2.2.2. Talent pipetting up and down to resuspend the cells in 1 milliliter of *Agrobacterium* induction medium.

2.2.3. Talent transferring the resuspended cells into a foil-covered 50 milliliter sterile tube.

2.2.4. Talent adding *Agrobacterium* induction medium.

2.2.5. Talent placing the sample in a sepctrophotmeter.

2.2.6. Talent adding *Agrobacterium* induction medium to reach OD600 of 0.4.

2.3. Then, loosen the lid of the foil-covered tube containing the bacterial suspension [1],

place it on a platform shaker with gentle agitation at approximately 70 revolutions per minute [2-TXT].

2.3.1. Talent loosening the lid of the foil-covered tube.

2.3.2. Talent placing the tube on a shaker set to 70 revolutions per minute. **TXT: RT; 1.5 - 2 h**

3. Collecting the Zoospores from *Phytophthora palmivora*

3.1. Flood a 7 to 8-day-old *Phytophthora palmivora* plate with 10 milliliters of water pre-cooled to 4 degrees Celsius [1]. Use a pipette tip to gently tap the surface and remove any air bubbles on the media [2]. Incubate the plate at 4 degrees Celsius for 15 minutes [3], then transfer it to room temperature under light for another 15 minutes [4].

3.1.1. Talent flooding the oomycete culture plate with 10 milliliters of pre-chilled water.

3.1.2. Talent using a pipette tip to gently tap and remove bubbles from the plate surface.

3.1.3. Talent placing the plate into a 4 degrees Celsius environment.

3.1.4. Talent transferring the plate to a shelf under light at room temperature.

3.2. Using a pipette, gently transfer approximately 7 to 8 milliliters of released zoospores into a sterile 15 milliliter centrifuge tube without disturbing the mycelia and sporangia [1].

3.2.1. Talent carefully pipetting 7 to 8 milliliters of the zoospore suspension into a sterile 15 milliliter centrifuge tube.

3.3. Then, transfer 20 microliters of the zoospore suspension to a 1.5 milliliter microcentrifuge tube [1] and add 180 microliters of water to dilute the suspension 10-fold [2]. Vortex the tube for 1 minute to encyst the zoospores [3] and use a hemacytometer under a microscope to count the zoospores [4].

3.3.1. Talent pipetting 20 microliters of zoospore suspension into a 1.5 milliliter microcentrifuge tube.

3.3.2. Talent adding 180 microliters of water to the microcentrifuge tube.

3.3.3. Talent vortexing the tube for 1 minute.

3.3.4. Talent placing the hemacytometer below a microscope.

4. Co-Incubation of Agrobacteria and Zoospores

4.1. Add 5 milliliters of the prepared *Agrobacterium* suspension to 5 milliliters of the zoospore suspension in a sterile 50-milliliter foil-covered tube [1]. Gently mix the contents [2], loosen the lid of the tube [3], and let it sit at room temperature for 2 hours [4].

4.1.1. Talent adding 5 milliliters each of Agrobacterium and zoospore suspension into a sterile 50 milliliter foil-covered tube.

4.1.2. Talent gently inverting the tube to mix the contents.

4.1.3. Talent loosening the lid of the tube.

4.1.4. Talent placing the tube in a rack at room temperature.

4.2. During the incubation, prepare the AZCA plates [1-TXT]. Once the medium solidifies, place one piece of sterilized Hybond N plus membrane onto the plate [2], and keep the plate in the dark [3].

4.2.1. Talent pouring AZCA medium plates in a hood. **TXT: AZCA: Agrobacterium-Zoospore Co-cultivation Agar**

4.2.2. Talent placing a sterilized Hybond N plus membrane on the surface of each solidified plate.

4.2.3. Talent covering the plates with a black plastic bag .

4.3. After 2 hours of co-incubation, use a pipette tip to spread 300 microliters of the mixture onto the Hybond N plus membrane, ensuring the margins are free from the liquid [1].

4.3.1. Talent pipetting 300 microliters of the mixture and gently spreading it across the membrane without touching the edges.

4.4. Leave the Petri dishes uncovered in the hood for about 10 minutes to allow the mixture to air-dry [1]. Once the liquid stops moving on the membrane, cover the plates [2].

4.4.1. Talent leaving the Petri dishes open in a hood for drying.

4.4.2. Talent covering the plates once the membrane surface is visibly dry and stable.

4.5. Incubate the covered plates at 25 degrees Celsius in the dark for 2 days [1].

4.5.1. Talent placing the plates in a 25 degrees Celsius incubator set to dark conditions.

5. Selection of G418-Resistant Transformants

5.1. After 48 hours of incubation, use sterilized forceps to transfer the Hybond N plus membrane upside-down onto the Plich agar plate without dragging it across the agar surface [1-TXT].

5.1.1. Talent lifting the membrane with sterile forceps and flipping it onto the Plich agar plate surface. **TXT: Supplement Plich agar with 30 µg/mL G418 and 100 µg/mL Cefotaxime**

5.2. Use the flat base of a 1.5-milliliter microcentrifuge tube to gently press and slide across the membrane surface to ensure full contact with the medium [1]. Check the underside of the plate for air bubbles [2], and gently press it out using the same flat base [3].

5.2.1. Talent pressing and sliding the flat base of a microcentrifuge tube across the membrane.

5.2.2. Talent holding up the plate to inspect for air bubbles beneath the membrane.

5.2.3. Talent gently pressing out visible air bubbles with the same tube base.

5.3. Incubate the plate at 25 degrees Celsius under a 12-hour light and 12-hour dark cycle for 2 to 3 days [1].

5.3.1. Talent placing the plate into an incubator.

5.4. Finally, after 2 to 3 days of incubation, remove the membrane using sterile forceps [1] and continue incubating the plate under the same light and temperature conditions [2].

5.4.1. Talent removing the membrane with sterile forceps.

5.4.2. Talent returning the plate to the incubator.

Results

6. Results

6.1. Co-incubation of *Phytophthora palmivora* zoospores with *Agrobacterium tumefaciens* EHA105 expressing pCB301TOR (*P-C-B-3-0-1-tor*)-GFP yielded G418-resistant colonies on Plich agar supplemented with 30 micrograms per milliliter G418 [1], whereas no colonies appeared on control plates without *Agrobacterium* infection [2].

6.1.1. LAB MEDIA: Figure 1A. *Video editor: Highlight the plate labeled "Transformed with Agrobacterium" showing visible fungal colonies.*

6.1.2. LAB MEDIA: Figure 1A. *Video editor: Highlight the plate labeled "Non-transformed control" showing no colonies.*

6.2. The number of G418-resistant transformants per 10^7 zoospores was 55 in experiment 1 [1] and 50 in experiment 2 [2].

6.2.1. LAB MEDIA: Table 1. *Video editor: Highlight the cell showing "55" in the row "Exp 1" and column "No. of G418-resistant colonies / 10^7 zoospores".*

6.2.2. LAB MEDIA: Table 1. *Video editor: Highlight the cell showing "50" in the row "Exp 2" and column "No. of G418-resistant colonies / 10^7 zoospores".*

6.3. Fluorescence microscopy confirmed a clear green fluorescent protein signal in *Phytophthora palmivora* transformants [1].

6.3.1. LAB MEDIA: Figure 1B. *Video editor: Highlight the lower-right GFP image of the "Transformant" panel showing bright green fluorescent dots.*

6.4. Additionally, this protocol was applied to *Phytophthora capsici* to successfully generate transformants expressing GFP [1].

6.4.1. LAB MEDIA: Supplemental Figure 1. *Video editor: Highlight the lower-right green GFP image for "Transformant" panel*

Pronunciation Guide:

Agrobacterium

Pronunciation link: <https://www.merriam-webster.com/dictionary/Agrobacterium>

IPA: /,ægroʊbæk'tɪriəm/

Phonetic Spelling: ag-roh-bak-teer-ee-uhm

Agrobacterium tumefaciens

Pronunciation link: <https://www.merriam-webster.com/dictionary/Agrobacterium>

IPA: /,ægroʊbæk'tɪriəm ,tu:mə'feɪʃi:ɛnz/

Phonetic Spelling: ag-roh-bak-teer-ee-uhm too-muh-fay-shee-enz

Transformation

Pronunciation link: <https://www.merriam-webster.com/dictionary/transformation>

IPA: /,trænsfər'meɪʃən/

Phonetic Spelling: trans-fer-may-shuhn

Phytophthora

Pronunciation link: <https://www.merriam-webster.com/dictionary/Phytophthora>

IPA: /faɪ'tɑ:fθərə/

Phonetic Spelling: fy-toff-thuh-ruh

Oomycetes

Pronunciation link: <https://www.merriam-webster.com/dictionary/oomycete>

IPA: /'oʊəmaɪ'si:ts/

Phonetic Spelling: oh-uh-my-seets

Microcentrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/microcentrifuge>

IPA: /,maɪkroʊ'sentrə,fju:dʒ/

Phonetic Spelling: my-kroh-sen-truh-fyooj

Spectrophotometer

Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>

IPA: /,spektrəfoʊ'tɑ:mətər/

Phonetic Spelling: spek-truh-foh-tom-uh-ter

Zoospores

Pronunciation link: <https://www.merriam-webster.com/dictionary/zoospore>

IPA: /'zoʊə,spɔ:rz/

Phonetic Spelling: zoh-uh-sporz

Mycelia

Pronunciation link: <https://www.merriam-webster.com/dictionary/mycelium>

IPA: /maɪ'si:liə/

Phonetic Spelling: my-see-lee-uh

Sporangia

Pronunciation link: <https://www.merriam-webster.com/dictionary/sporangium>

IPA: /spəˈrændʒiə/

Phonetic Spelling: spuh·ran·jee·uh

Hemacytometer

Pronunciation link: <https://www.merriam-webster.com/dictionary/hemacytometer>

IPA: /ˌhiːməsaɪˈtɑːmɛtər/

Phonetic Spelling: hee·muh·sy·tom·uh·ter

Encyst

Pronunciation link: <https://www.merriam-webster.com/dictionary/encyst>

IPA: /ɛnˈsɪst/

Phonetic Spelling: en·sist

Co-incubation

Pronunciation link: <https://www.merriam-webster.com/dictionary/coincubation>

IPA: /ˌkoʊɪnkjəˈbeɪʃən/

Phonetic Spelling: koh·in·kyuh·bay·shuhn

Cefotaxime

Pronunciation link: <https://www.merriam-webster.com/dictionary/cefotaxime>

IPA: /ˌsefoʊˈtæksiːm/

Phonetic Spelling: sef·oh·tak·seem

Transformants

Pronunciation link: <https://www.merriam-webster.com/dictionary/transformant>

IPA: /trænsˈfɔːrmənt/

Phonetic Spelling: trans·for·munt

Fluorescence

Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>

IPA: /flʊˈresəns/

Phonetic Spelling: floor·es·uhns

Fluorescent

Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescent>

IPA: /flʊˈresənt/

Phonetic Spelling: floor·es·uhnt

Capsici

Pronunciation link: No confirmed link found

IPA: /ˈkæpsɪˌsaɪ/

Phonetic Spelling: kap·sih·sy

Reproducible

Pronunciation link: <https://www.merriam-webster.com/dictionary/reproducible>

IPA: /ˌriːprəˈduːsəbəl/

Phonetic Spelling: ree-pruh-doo-suh-buhl