FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 67729
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=20657393

Title: Optimized Method for Cultivation and Microbial Bioaugmentation of *Typha latifolia* (Cattail)

Authors and Affiliations:
Ann Zymela, Douglas Muench

Department of Biological Sciences, University of Calgary

Corresponding Authors:
Douglas Muench (dmuench@ucalgary.ca)
Email Addresses for All Authors:
Ann Zymela (ann.zymela@ucalgary.ca)
Douglas Muench (dmuench@ucalgary.ca)

Author Questionnaire

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

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

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes, Maybe 10 to 15 min drive**

Current Protocol Length
Number of Steps: 24
Number of Shots: 55

Introduction

Videographer: Obtain headshots for all authors available at the filming location.
Videographer's Note: Clip0002 – Grey Card/White Card for interview space

1.1. **Annie Zymela:** Our work addresses the lack of protocols for growing cattail from seed. We developed reproducible methods for seed germination, early growth, and microbial bioaugmentation to support future plant-microbe research.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:6.1*

Videographer's Note: Clip0005 – WS of 1.1.1 and 1.5.1, Clip0006 – MS of 1.1.1 and 1.5.1, Clip0007 – Head Shot of Annie Zymela

What are the most recent developments in your field of research?

1.2. **Annie Zymela:** Few studies grow cattail from seed or track them to maturity, yet cattails are commonly used in wetland remediation research.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:5.4*

What technologies are currently used to advance research in your field?

1.3. **Annie Zymela:** Most cattail research involves purchasing rhizomes for propagation of cattails in laboratory studies.

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

What are the current experimental challenges?

1.4. **Annie Zymela:** Few studies grow cattail from seed, leaving no standardized methods. Achieving consistent germination and persistent colonization of introduced microbes has been a major challenge.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:6.3*

What significant findings have you established in your field?

1.5. **Annie Zymela:** We've developed reproducible methods for seed germination and microbial bioaugmentation, demonstrating how early inoculation helps support long term colonization.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:6.2*

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Scarification of *Typha latifolia* Seeds

Demonstrator: Annie Zymela

2.1. To begin, use garden shears to cut the stem of a *Typha latifolia* plant approximately 2 centimeters from the base of the inflorescence **[1]**. Pull the seeds off the inflorescence **[2].** Transfer the seed into a laboratory blender until the blender is approximately one-fourth full corresponding to about 250 milliliters of uncompacted seeds **[3]**.

2.1.1. WIDE: Talent using garden shears to cut the stem of the plant just below the inflorescence.
Videographer's Note: Clip0010 – 2.1.1a – Optional additional shot of talent walking into lab,
Clip0072 – NO SLATE – 2.1.1
Clip0073 – NO SLATE – 2.1.1 alternative

2.1.2. Talent pulling seeds from the inflorescence.

2.1.3. Shot of the seeds being transferred into a laboratory blender up to the one-fourth mark.

2.2. Now, fill the blender with 500 milliliters of tap water, ensuring a 10-centimeter headspace is maintained **[1]**. Blend the mixture at medium-low speed for 20 seconds and immediately transfer the viscous contents into a 1-liter beaker **[2]**.

2.2.1. Talent filling the blender with tap water and leaving space at the top.

2.2.2. Talent operating the blender and pouring the viscous solution into a 1-liter beaker.

2.3. Transfer approximately 100 milliliters of the seed-water mixture back into the blender **[1]**. Then add 400 to 600 milliliters of fresh tap water and blend on medium-high speed for 20 seconds **[2].** Pour the contents into a fresh 1-liter beaker **[3]**.

2.3.1. Talent pouring 100 milliliters of the previous blend into the blender.

2.3.2. Shot of 400 – 600 mL water being added to the blender and the blender is being turned on.

2.3.3. Shot of the blended mix being poured into another beaker.

2.4. Now fill the beaker with tap water up to 800 milliliters **[1].** After letting it sit for 60 seconds, scoop off the floating sludge from the top without disturbing the seeds settled at the bottom **[2]**. Slowly pour out the remaining water and transfer the seeds into a 100-milliliter beaker **[3]**.

2.4.1. Talent filling beaker with water and allowing the mixture to settle.

2.4.2. Talent carefully scooping off the top layer of sludge.
Videographer's Note: Clip0017 – 2.4.2 – Take 1 – NG, Clip0018 – 2.4.2 – Take 2 – Good

2.4.3. Talent pouring off excess water and transferring seeds into a smaller beaker.
Videographer's Note: Clip0019 – 2.4.3 – Only the talent pouring out excess water, Clip0020 – 2.4.3a – Transferring seeds into small beaker

2.5. Repeat the blending process for the remaining seed-water sludge **[1]**.

2.5.1. Talent repeating the blending and separation process on the remaining mixture.

2.6. Next, place the beaker containing the separated seeds onto a stir plate **[1-TXT].** After stirring, scoop off any floating plant material from the surface of the beaker **[2]**.

2.6.1. Talent setting the beaker on a stir plate and starting the stir function.**TXT: Stir for 1 h at medium speed**

2.6.2. Talent using a scoop to remove floating debris from the top.

2.7. Pour the seeds into a Büchner funnel with filter paper attached to a vacuum and allow them to dry overnight **[1]**. Store the dried seeds at minus 20 degrees Celsius in 15-milliliter conical polypropylene tubes **[2]**.

2.7.1. Talent transferring seeds to a Büchner funnel setup under vacuum.

2.7.2. Talent placing dried seeds into labeled polypropylene tubes and storing them in a freezer.

3. *Typha latifolia* Seed Germination Using Sterile Technique

3.1. Prepare half-strength Murashige and Skoog media plates with 1% phytoagar **[1]**. Transfer approximately 1 milligram of the dried seeds into a 15-milliliter conical polypropylene tube **[2].** Then add 10 milliliters of sterile double-distilled water to the tube **[3]**. Place the tube on an orbital shaker at medium-high speed for 10 minutes **[3]**.

3.1.1. Shot of prepared MS media plates.

3.1.2. Talent pipetting seeds into a conical tube.

Videographer's Note: Clip0027 – 3.1.2 – *Note from author:* Action is not with pipette but with a scoop

3.1.3. Talent adding sterile water to the tube.

3.1.4. Talent placing the tube on an orbital shaker and turning it on.

3.2. Now, remove the water from the tube **[1]** and add 5 milliliters of 0.1% Polysorbate 20 solution prepared in sterile double-distilled water **[2]**. Shake the tube at medium-high speed for 10 minutes **[3]**.

3.2.1. Talent decanting or aspirating water from the conical tube.
Videographer's Note: Clip0030 – 3.2.1 – Only decanting in this show, Clip0031 – 3.2.1a – Aspirating water from conical tube

3.2.2. Talent adding 5 milliliters of Polysorbate 20 solution.

3.2.3. Talent placing the tube on a shaker.

3.3. Remove the Polysorbate 20 solution **[1].** Perform all further steps in front of a flame or laminar flow hood **[2].** Replace the solution with 5 milliliters of 30% commercial bleach **[3]** and 0.025% Polysorbate 20 solution prepared in sterile double-distilled water **[4-TXT]**.
NOTE: VO has been edited as per author's request. The shot numbers have been changed to accommodate the added shot

3.3.1. Talent removing the previous solution from the tube.
Added shot: 3.3.1a
Videographer's Note: Clip0036 – 3.3.1a – NEW ADDED SHOT: Happens before 3.3.2

3.3.2. Talent adding the bleach to the tube.
Videographer's Note: Clip0035 – 3.3.2 – Take 1 – NG, Clip0037 – 3.3.2 – Take 2 – Good

3.3.3. Shot of 0.025% Polysorbate 20 solution being added to the tube. **TXT: Shake tube at medium high speed for 30 min**

3.4. Replace the supernatant with sterile double-distilled water **[1]**. Then shake the tube at medium-high speed for 5 minutes **[2-TXT]**.

3.4.1. Talent decanting the bleach mixture and adding fresh sterile water.

3.4.2. Shot of the tube in a shaker. **TXT: Repeat water rinse 2 more times**

3.5. ~~Next, fill the 15-milliliter conical tube with sterile double-distilled water **[1]**.~~ After the

third rinse, rotate the tube at low speed for 24 hours to induce germination **[2]**. The next day, remove the excess water and ensure 3 milliliters of liquid remain in the tube **[3]**.

3.5.1. ~~Shot of sterile double distilled water being added to the tube~~.
NOTE: Shot deleted at author's request

3.5.2. Talent placing the tube on a low-speed rotator and starting the incubation.

3.5.3. Talent removing excess water from the tube, leaving only 3 milliliters.

3.6. Now, aseptically cut the tip of a 1000-microliter plastic pipette tip **[1]**. Use the cut pipette tip to vigorously pipette and suspend the seeds within the tip **[2]**.

3.6.1. Talent using a sterile blade to cut the pipette tip.

3.6.2. Talent using the modified pipette tip to mix and suspend the seeds.

3.7. Plate the seeds and liquid onto one-half strength Murashige and Skoog agar plates **[1]**. Gently swirl the plate to evenly distribute the seeds **[2]**.

3.7.1. Talent pipetting the seed suspension onto the MS plate.

3.7.2. Talent gently swirling the plate for even seed spread.

3.8. Wrap the plates with laboratory sealing film **[1]**. Then place them in a growth chamber with a 16-hour light and 8-hour dark cycle at 23 degrees Celsius and 70% humidity **[2-TXT]**.

3.8.1. Talent wrapping the agar plates with sealing film.

3.8.2. Talent placing the wrapped plates inside a growth chamber. **TXT: Leave plates for 1 - 2 weeks for germination**

4. *Typha latifolia* Seed Inoculation with Selected Bacteria

4.1. To inoculate cattail seeds, sterilize seeds with Polysorbate 20, bleach and double distilled water as demonstrated **[1]**. ~~Remove the final rinse water from the 15-milliliter conical polypropylene tube, ensuring that 3 milliliters of water remain **[2]**.~~

4.1.1. Shot of labeled tube with seeds and 3 mL sterile water.

4.1.2. ~~Talent removing excess water from the tube, leaving a small volume.~~
NOTE: Shot deleted at author's request

4.2. Measure the optical density of an overnight culture of *Luteimonas* isolate at 600 nanometers **[1]**. Normalize the culture to an absorbance value of 1.0 by diluting in

culture medium **[2-TXT]**.

4.2.1. Talent pipetting culture into a cuvette and placing it in a spectrophotometer.
Videographer's Note: Clip0050 – 4.2.1 – Only action of pipetting and into cuvette,
Clip0051 – 4.2.1a – Placing on spectrophotometer **Do not let results on monitor to be seen

4.2.2. Talent diluting culture with culture medium. **TXT: If necessary, incubate cultures longer until required density is achieved**
Videographer's Note: Re-use 4.1.1 for 4.2.2, and move that step after 4.3.1a

4.3. Now, centrifuge 1 milliliter of the culture at 9300 *g* for 2 minutes **[1]**. Remove the supernatant and resuspend the pellet in 1 milliliter of PBS **[2]**. After centrifuging and removing the supernatant again, resuspend the bacterial pellet in 1 milliliter of sterile double-distilled water **[3]**.

4.3.1. Talent pipetting microbial culture into a microcentrifuge tube and placing it in centrifuge.
Videographer's Note: Clip0053 – 4.3.1 – Pipetting culture in micro centrifuge, Clip0054 – 4.3.1a – Placing in centrifuge

4.3.2. Talent removing supernatant and adding phosphate-buffered saline to the pellet.
4.3.2a – Added shot - Centrifuge so we can see new pellet
Videographer's Note: Clip0056 for 4.3.2a, Clip0057 – No Slate – ECU of new pellet

4.3.3. Talent pipetting sterile water into the tube.

4.4. ~~Transfer 1 milliliter of the seeds to a 15-milliliter conical polypropylene tube **[1].**~~ Use sterilized seeds then dilute the inoculum at a 1 to 10 dilution with 0.025% organosilicone surfactant, in the same tube **[2].** Shake the suspension at low speed for 24 hours before seed germination **[3].**

~~4.4.1. Talent adding 1 mL seeds to a 15 mL tube.~~
NOTE: Shot deleted by authors~~-~~

4.4.2. Shot of the bacterial inoculum and organisilicone surfactant being added to the same tube.

4.4.3. Shot of the suspension being placed on a shaker at low speed.

5. *Typha latifolia* Seedling Growth

5.1. Start by filling a sterile plant growth chamber unit with a soil of choice pre-wetted with tap water **[1]**. Cover the Luer lock tip with aluminum foil **[2]** and autoclave the unit on

a liquid cycle for 20 minutes **[3-TXT]**.

5.1.1. Talent adding soil and water into the chamber unit.
Videographer's Note: Clip0061 – 5.1.1 – Only adding soil

5.1.2. Talent wrapping the tip with foil.
Videographer's Note: Clip0062 – 5.1.2 – Include action of adding water into chamber

5.1.3. Talent placing the unit into the autoclave. **TXT: Repeat autoclaving after 24 h**

5.2. Next, in a laminar flow hood, attach a 0.2-micrometer filter unit to the Luer lock connector on the growth chamber **[1]**. Open the chamber and add 500 microliters of filter-sterilized 1% 20/20/20 *(Twenty-by-twenty-by-Twenty)* fertilizer to the soil **[2]**.

5.2.1. Talent attaching the filter to the Luer lock port.

5.2.2. Talent opening the chamber and adding 500 µL of filter sterilized 1% 20/20/20 fertilizer.

5.3. Mix the soil with a sterile spatula while simultaneously adding sterile double-distilled water to maintain hydration without oversaturating the soil **[1]**.

5.3.1. Talent gradually adding water while stirring the soil to moisten it appropriately.

5.4. Now, use a sterile razor blade to cut Murashige and Skoog agar from plates containing 1-week-old seedlings into quarters **[1]**. With a sterile spatula, transfer one agar section with seedling onto the prepared soil in the growth chamber **[2]**.

5.4.1. Talent slicing agar plate into quarters using a sterile blade.

5.4.2. Talent using sterile spatula to place agar section with seedling into soil.
Videographer's Note: Clip0068 – 5.4.2 – Take 1 – Some parts of this clip are not good, Clip0069 – Mislabelled slate – Should be 5.4.2 – Take 2 – Good

5.5. Transfer the growth chamber unit into a plant growth incubator set to a 16-hour light and 8-hour dark cycle at 23 degrees Celsius and 70% humidity **[1]**.

5.5.1. Talent placing the sealed growth chamber into the plant incubator and adjusting settings on control panel.

Results

6. Results

6.1. Complete scarification of *Typha* seeds resulted in visibly separated seed components **[1]**, whereas incomplete scarification left the beak attached, and non-scarified seeds remained intact **[2].**

6.1.1. LAB MEDIA: Figure 1B. *Video editor: Highlight seed number 1 showing the separated plume, beak, and seed.*

6.1.2. LAB MEDIA: Figure 1B. *Video editor: Highlight seeds number 2 and 3*

6.2. The sterile hydroponic growth system successfully supported the establishment and growth of *Juncus* species seedlings up to 1 year **[1].**

6.2.1. LAB MEDIA: Figure 2B.

6.3. The highest seed germination rate of 20.8% was observed using the 30% bleach and Polysorbate 20 method **[1]**, which was significantly greater than all other sterilization treatments except for the 1-hour chlorine gas method **[2].**

6.3.1. LAB MEDIA: Figure 3. *Video editor: Highlight the tallest bar labeled "30% bleach and Polysorbate 20". Use file Fig 3 ed.jpg*

6.3.2. LAB MEDIA: Figure 3. *Video editor: Highlight the remaining bars*

6.4. At 7 days post-scarification, germinating *Typha* seedlings developed visible radicles and shoot tissue in the non-sterile Petri plate environment **[1]**. Following transplant to soil, a subset of *Typha* seedlings established successfully and produced new shoot tissue within 1 to 2 weeks **[2]**.

6.4.1. LAB MEDIA: Figure 4A.

6.4.2. LAB MEDIA: Figure 4B

6.5. After inoculation with *Luteimonas* species carrying a DsRed *(D-S-Red)* plasmid, red fluorescent bacterial colonization was observed throughout the *Typha* seedling roots at 16 days post-inoculation **[1]**.

6.5.1. LAB MEDIA: Figure 5A and B . *Video editor: Highlight the red fluorescent dots*

Pronunciation Guide:

1. *Typha latifolia*
Pronunciation link: https://www.howtopronounce.com/typha-latifolia pronouncekiwi.com+15How To Pronounce+15How To Pronounce+15
IPA: /ˈtaɪfə lætəˈfoʊliə/
Phonetic Spelling: TYE-fuh lat-uh-FOE-lee-uh

---

2. *bioaugmentation*
Pronunciation link: https://www.howtopronounce.com/bioaugmentation How To PronounceSynonyms.com
IPA: /ˌbaɪoʊˌɔːgmənˈteɪʃən/
Phonetic Spelling: bye-oh-awg-mən-TAY-shuhn

---

3. *Murashige and Skoog* (as in "Murashige and Skoog medium")
Pronunciation link: https://www.howtopronounce.com/murashige-e-skoog How To PronounceHow To Pronounce
IPA: /ˌmʊrəˈʃiːgeɪ ænd skoʊg/
Phonetic Spelling: moo-ruh-SHEE-gay and skohg

---

4. *Büchner funnel*
Pronunciation link: https://www.merriam-webster.com/medical/B%C3%BCchner%20funnel Merriam-WebsterCambridge Dictionary
IPA: /ˈbuːxnər ˌfʌnəl/
Phonetic Spelling: BOOCH-ner FUHN-uhl

---

5. *Luteimonas*
Pronunciation link: No confirmed link found via Merriam-Webster or Oxford. HowToPronounce did not have entries. en.wikipedia.orgfindwords.info
IPA (based on genus Latin pronunciation): /ˌluːtiːˈmoʊnəs/
Phonetic Spelling: loo-tee-MOH-nuhs