FINAL SCRIPT: APPROVED FOR FILMING

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Title: Quantification of Violacein in *Chromobacterium violaceum* and its Inhibition by Bioactive Compounds

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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? **No**

Current Protocol Length
Number of Steps: 16
Number of Shots: 28

Introduction

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

1.1. **Uelinton Manoel Pinto:** Our research aims to identify compounds that inhibit quorum sensing, which regulates key phenotypes. This protocol screens molecules that reduce violacein production without affecting growth, suggesting quorum sensing interference.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

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

1.2. **Uelinton Manoel Pinto:** Recent studies have identified new bioactive compounds that inhibit quorum sensing without affecting bacterial growth, reinforcing their potential as antivirulence agents against antibiotic-resistant bacteria.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

What are the current experimental challenges?

1.3. **Catarina Angeli Santos:** Main challenges include specificity of quorum sensing inhibition, since compounds may affect growth or other traits. In this protocol, color interference and solubility impact violacein quantification and require careful handling.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1*

What significant findings have you established in your field?

1.4. **Emília Maria França Lima:** We established a protocol using bioactive compounds at sub-inhibitory concentrations, that do not affect bacterial growth. This is a key premise for studying QS signaling, different from antimicrobial research.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.2.1*

What research questions will your laboratory focus on in the future?

1.5. **Cristina Miguel:** We will focus on identifying new quorum sensing inhibitors and elucidating the inhibition mechanisms of this communication, especially in bacteria relevant to food and health.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.4.1*

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

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Uelinton Manoel Pinto, Associate Professor at the Department of Food and Experimental Nutrition, School of Pharmaceutical Sciences, University of São Paulo.** (authors will present their testimonial statements live)

I believe publishing in JoVE will boost the visibility of our lab in the field. Additionally, through video demonstration, we expect to have better reproducibility for assessing quorum sensing inhibitors, allowing better comparison between different studies. I think the format attracts microbiologists and researchers in the field of drug-discovery, which likely will increase citations of our works and enhance the chances of collaboration with other groups.

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

Protocol

2. Culture and Compound Preparation

Demonstrator: Cristina Miguel

2.1. To begin, grow a 5-milliliter culture of *Chromobacterium violaceum* ATCC 12472 *(1-2-4-7-2)* in Luria Bertani or LB broth **[1]**. Incubate the culture at 30 degrees Celsius in a shaking incubator set at 220 revolutions per minute for 24 hours **[2]**.

2.1.1. WIDE: Talent pipetting 5 milliliters of *C. violaceum* into a test tube containing Luria Bertani broth.

2.1.2. Talent placing the culture tube into a shaking incubator.

2.2. Measure the optical density of the overnight culture **[1]** and adjust it to approximately 1×10^8 colony-forming units per milliliter using the LB broth **[2]**.

2.2.1. Talent placing the culture in the spectrophotometer.

2.2.2. Talent adding the culture with fresh Luria Bertani broth.

2.3. For compound preparation, weigh the bioactive compound **[1]** and dilute it in either pure DMSO or a 1 to 1 mixture of DMSO and water, depending on the solubility **[2].** Take a flat-bottom 96-well plate **[3]** and add LB broth along with the bioactive compound according to a serial dilution scheme **[4]**. NOTE: The VO is edited for the additional shots

Added shot: 2.3.0a. Talent weighing the compound on the scale

Added shot: 2.3.0b. Talent adding DMSO to the tube and mixing it on the shaker

~~2.3.1. Talent flicking the tube with compound to mix.~~ NOTE: Not filmed

2.3.2. Talent placing a 96-well plate. NOTE: This shot is the same as 3.1. Reuse 3.1.1

2.3.3. Talent pipetting Luria Bertani broth into each well of a flat-bottom 96-well plate using a multichannel pipette.

2.4. To calculate the required volume of stock solution for the desired concentration in each well, apply the formula **[1-TXT]**.

2.4.1. Talent writing something in a notebook. **TXT: $C_1V_1 = C_2V_2$**

3. Serial Dilution and Plate Preparation

Demonstrator: Catarina Angeli Santos

3.1. In the first column of each serial dilution set, add 196 microliters of Luria Bertani broth to columns 3, 5, and 7 **[1]**. Then, in the subsequent columns used for the dilution, add 100 microliters of Luria Bertani broth to columns 4, 6, and 8 **[2]**.

3.1.1. Talent pipetting 196 microliters of Luria Bertani broth into wells in columns 3, 5, and 7 of a 96-well plate.

3.1.2. Talent pipetting 100 microliters of Luria Bertani broth into wells in columns 4, 6, and 8.

3.2. To initiate the serial dilution, add 4 microliters of the bioactive compound to the first well of each set, which corresponds to columns 3, 5, and 7 **[1]**.

3.2.1. Talent pipetting up and down to mix the bioactive compound in columns 3, 5, and 7.

3.3. After mixing, transfer 100 microliters from each well to the adjacent column to continue the serial dilution, reaching columns 4, 6, and 8 **[1-TXT]**.

3.3.1. Talent transferring the mix into the adjacent columns. **TXT: Repeat the mixing and transfer to attain the final desired concentration**

3.4. Repeat the entire procedure in triplicate for each concentration of every compound using fresh tips to avoid cross-contamination **[1]**.

3.4.1. Talent labelling 3 replicate plates.

3.5. Add 80 microliters of Luria Bertani broth to rows B through D in all wells, bringing the total to 180 microliters per well **[1-TXT]**. Then, add 20 microliters of the standardized *Chromobacterium violaceum* culture from the previous preparation to each of those wells **[2-TXT]**, reaching a final volume of 200 microliters **[3]**.

3.5.1. Talent adding 80 microliters of Luria Bertani broth to each well in rows B to D of the 96-well plate. **TXT: Change pipette tips between each well**

3.5.2. Talent pipetting 20 microliters of standardized *C. violaceum* culture into well in rows B to D. **TXT: Control: LB broth (no bacteria); Fill empty wells with media/broth**

3.5.3. LAB MEDIA: Figure 3

4. Incubation, Extraction, and Quantification of Violacein

Demonstrator: Emília Lima

4.1. Based on the spectrophotometer compatibility, cover the microplate with a lid or sealing film to prevent evaporation **[1]**.

4.1.1. Talent sealing the 96-well plate with a lid.

4.2. Incubate the sealed plate at 30 degrees Celsius with agitation at 130 revolutions per minute for 24 hours **[1]**. After incubation, transfer the plate to a drying incubator set at 60 degrees Celsius **[2]**, remove the lid, and allow it to dry completely for approximately 8 hours **[3]**.

4.2.1. Talent placing the sealed microplate in an orbital shaker incubator set at 30 degrees Celsius and 130 revolutions per minute.

4.2.2. Talent transferring the incubated plate to a drying incubator at 60 degrees Celsius.

4.2.3. Talent removing the plate's lid.

4.3. Once dry, remove the plate from the incubator and add 200 microliters of pure DMSO to each well **[1]**. Cover the plate **[2]** and incubate it at 25 degrees Celsius with shaking at 130 revolutions per minute for 30 minutes to dissolve the violacein pigment **[3]**.

4.3.1. Talent pipetting 200 microliters of pure dimethyl sulfoxide into each well of the dried microplate.

4.3.2. Talent closing the plate lid.

4.3.3. Talent placing the plate into an orbital shaker incubator set at 25 degrees Celsius and 130 revolutions per minute.

4.4. After incubation, carefully transfer 100 microliters of the dissolved violacein solution to a new microplate, avoiding contact between the pipette tip and the well walls **[1]**.

4.4.1. Talent transferring 100 microliters from each well of the original plate to a new microplate, pipetting carefully without touching the well sides.

4.5. Using a microplate spectrophotometer, measure the absorbance of violacein. Input the parameters displayed on the screen into the spectrophotometer software. Set the temperature to 37 degrees Celsius with a 2 degrees Celsius gradient. Take readings every 2 hours for 24 hours, shake for 5 seconds before each reading **[1],** and perform

the reading with the lid on to avoid contamination **[2].** NOTE: The VO is edited for the additional shot

Added shot: Violacein-reading-video.mkv: 00:08-01:08

4.5.1. Talent placing the plate in a microplate spectrophotometer.

4.6. To calculate violacein production, first subtract the absorbance of the blank from each sample to exclude background interference **[1]** and then, calculate the percentage of violacein using the untreated control as the reference with the equation **[2-TXT]**.

4.6.1. Shot of the talent working in a computer with a spreadsheet displayed on the monitor.

4.6.2. Shot of the computer monitor displaying the formula and the calculated values side by side. **TXT: Perform statistical analysis** Authors, you may point out the final values

Results

5. Results

5.1. Three bioactive compounds-resveratrol, farnesol, and linalool were tested for their ability to inhibit violacein production in *Chromobacterium violaceum* ATCC 12472 **[1]**.

5.1.1. LAB MEDIA: Figure 4

5.2. Farnesol significantly reduced violacein production to 9% at 200 micrograms per milliliter **[1]** and to 19% at 100 micrograms per milliliter **[2]**, compared to 100% in the untreated control **[3]**.

5.2.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the bar labeled "200 µg/mL" on the left side of the graph where the bar is shortest and colored light blue*.

5.2.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the bar labeled "100 µg/mL" in the middle of the graph where the bar is slightly taller and colored medium blue.*

5.2.3. LAB MEDIA: Figure 4A. *Video editor: Highlight the bar labeled "Untreated control" on the right side of the graph where the bar is tallest and dark blue*.

5.3. Linalool treatment at both 200 and 100 micrograms per milliliter resulted in violacein production of 32%, showing a 68% inhibition **[1]** relative to control **[2]**.

5.3.1. LAB MEDIA: Figure 4B. *Video editor: Highlight both bars labeled "200 µg/mL" and "100 µg/mL"*.

5.3.2. LAB MEDIA: Figure 4B. *Video editor: Highlight "untreated control" bar*.

5.4. Resveratrol at 25 micrograms per milliliter reduced violacein production to 15% **[1]**, while at 12 micrograms per milliliter it reached 30% **[2]**, compared to 100% in the untreated control **[3]**.

5.4.1. LAB MEDIA: Figure 4C*. Video editor: Highlight the bar labeled "25 µg/mL" on the left side of the graph where the bar is shortest and light purple*.

5.4.2. LAB MEDIA: Figure 4C. *Video editor: Highlight the bar labeled "12 µg/mL" in the middle of the graph where the bar is taller and medium purple*.

5.4.3. LAB MEDIA: Figure 4C. *Video editor: Highlight the bar labeled "Untreated control" on the right side of the graph where the bar is tallest and dark purple*.

5.5. Microplate images visually confirmed reduced violacein pigmentation for all treatments **[1]** compared to the untreated control **[2]**.

5.5.1. LAB MEDIA: Figure 4. *Video editor: In all three image panels to the right of each graph, highlight the first 2 columns of wells with fading color*.

5.5.2. LAB MEDIA: Figure 4 *Video editor: In all three image panels to the right of each graph, highlight "Control" wells*.

Chromobacterium

- **Pronunciation link (Merriam-Webster Medical)**: https://www.merriam-webster.com/medical/chromobacterium
- **IPA (American English)**: /ˌkroʊmoʊbækˈtɪriəm/
- **Phonetic Spelling**: kroh-moh-bak-TEER-ee-uhm

Violacein

- **Pronunciation link**: No confirmed link found (not listed in Merriam-Webster)
- **IPA (Approximate American English)**: /ˌvaɪəˈleɪsɪn/
- **Phonetic Spelling**: vy-uh-LAY-sin

Pipette

- **Pronunciation link (Merriam-Webster)**: https://www.merriam-webster.com/dictionary/pipette *(Note: you may need to switch the query to pipette explicitly, but entry is there.)*
- **IPA (American English)**: /paɪˈpɛt/
- **Phonetic Spelling**: pie-PET

Broth

- **Pronunciation link (Merriam-Webster)**: https://www.merriam-webster.com/dictionary/broth
- **IPA (American English)**: /brɔθ/ or /brɑθ/
- **Phonetic Spelling**: broth (rhymes with "cloth")

Bacteria

- **Pronunciation link (Merriam-Webster)**: https://www.merriam-webster.com/dictionary/bacteria
- **IPA (American English)**: /bækˈtɪəriə/
- **Phonetic Spelling**: bak-TEER-ee-uh

Luria *(as in "Luria Bertani broth")*

- **Pronunciation link**: No confirmed link found (not in Merriam-Webster)
- **IPA (Estimated, common American usage)**: /ˈlʊriə/
- **Phonetic Spelling**: LOO-ree-uh

Dilution

- **Pronunciation link**: No confirmed link found (not requested earlier, but a technical term)
- **IPA (American English)**: /daɪˈluʃən/
- **Phonetic Spelling**: dye-LOO-shuhn

Sealing (as in “sealing film”)

- **Pronunciation link**: No confirmed link found
- **IPA (American English)**: /ˈsiːlɪŋ/
- **Phonetic Spelling**: SEE-ling