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We are still developing and optimizing this protocol

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Compound Screening and Growth Curves

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DISCLAIMER

This protocol was created for the BIT 479/579 High-throughput Discovery course and for use with students. It has been used by and optimized for students.

ABSTRACT

Overview

The bacterial genus *Delftia* was named for the city of Delft in the Netherlands, where the species type was isolated and for the Delft research groups that had a critical role in the early development of bacteriology. Species of *Delftia* have since been isolated all over the world in different environments and have been known to cause infections in humans.[1] *Delftia* are gram-negative rod-shaped aerobes. Species of Delftia are often resistant to several compounds, including the common disinfectant chlorhexidine,[2] most antibiotics in the aminoglycoside group,[3] and heavy metals.[4] One of *Delftia's* most surprising talents is its ability to concentrate heavy metals like gold.[5] Biofilms found on gold deposits suggest that these bacteria are responsible for their formation.[6] Known species of *Delftia* include *D. acidovorans, D. tsuruhatensis, D. deserti, D. lucustris,* and *D. litopenaei*. Refer to **Figure 1** for an example of *Delftia acidovorans* colonies growing on tryptic soy agar (TSA).

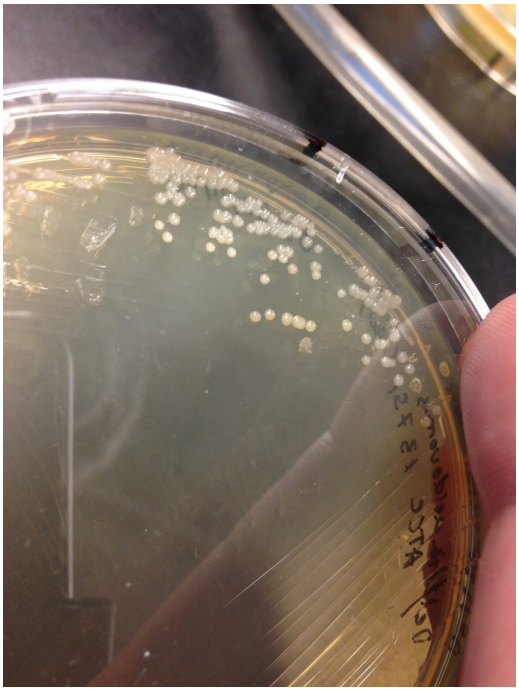

Figure 1. *Delftia acidovorans* colonies growing on tryptic soy agar (TSA)

Where are Delftia spp. found?

Delftia spp. are commonly isolated from aquatic environments such as lakes[7] and wastewater[8] and are thought to be ubiquitous. Because of their affinity for aquatic environments, Delftia have been identified in organisms and objects frequently found in these environments, such as mosquitoes9 and birds' feathers.[10] *Delftia* aren't just aquatic microbes, though. These bacteria have been identified in unexpected places like desert soil[11] and contact lens cases.[12]

An Opportunistic Pathogen

Species of *Delftia* have been known to cause various infections in healthy and immunocompromised people. Many of these infections are (IV) line-related,13,14 but other infections have occurred due to intravenous drug use.[3] *Delftia* were also implicated in eye inflammation related to contact lens use.[12] NC State students and staff have even found *Delftia* DNA in surveys of kitchen sinks performed by students in the BIT 477/577 Metagenomics lab module!

Our Project

We will set up a series of high-throughput (HT) assays to test whether different gold chloride concentrations inhibit the growth of different *Delftia* strains. Gold chloride has been explored for its antibacterial properties[15]. However, *Delftia* spp. have a unique non-ribosomal peptide that helps relieve gold toxicity by producing

delftibactin[5].
Bacterial strains will be grown in 96-well plates to test multiple concentrations of gold chloride and antibiotics and the effect of **miniaturization** of the assay. We will set up plates in the first lab session to test several strains at multiple doses with numerous replicates. Plates will incubate for one day at 30ºC before assessing cell viability using a stain for metabolic activity, PrestoBlue®. This approach can be adapted to screen active compounds from a high-throughput screening (**HTS**) campaign. For this first compound screen, we will focus on the **effects of automation and miniaturization on assay reproducibility.**

We will test gold chloride concentrations in a twelve-point dose-response curve. At the end of this experiment, **you can determine which concentrations are inhibitory by calculating the percent growth inhibition and IC_{50}.** We will compare the susceptibility of various *Delftia* strains.

IMAGE ATTRIBUTION

Delftia acidovorans ATCC 13751 colonies grown on tryptic soy agar (TSA). NC State University Biotechnology Program (BIT). William DeFoor, BIT SURE 2014.

GUIDELINES

Additional Resource : PrestoBlue Cell Viability Reagent Protocol

MATERIALS

Materials

- Overnight cultures of *Delftia acidovorans* SPH-1, *Delftia acidovorans* Cs1-4, and other *Delftia* spp. isolates in 2.5 ml of Tryptic Soy Broth (TSB, 30ºC with shaking 16-18 hrs)
- Teknova™Tryptic Soy Broth (TSB)
- Corning® 96 Well Clear Flat Bottom TC-Treated Microplate (Corning 3628)
- PrestoBlue® viability stain (Thermo Fisher A13261)
- epMotion 5075 and 50 and 300 filter tips (Eppendorf)
- 30 ml reservoirs (sterile, Eppendorf 960051009)
- Gold chloride solution 200 mg/dL in deionized water from Sigma-Aldrich (MFCD00011322)
- Teknova antibiotic stocks

Equipment

- Eppendorf epMotion 5075 liquid handler
- Eppendorf BioSpec
- Biotek/Agilent LogPhase 600 plate reader

SAFETY WARNINGS

While *Delftia* spp. are ubiquitous, we will treat them as potential sources of infection and abide by BSL-2 standards. We will minimize contact with cultures, use hoods, and submit waste for disinfection/autoclaving. Please use the clear biohazard bags at your stations and the biohazard bin next to the sink.

BEFORE START INSTRUCTIONS

Please put on your personal protective equipment (PPE) supplied by your instructors. PPE for this lab includes gloves, disposable lab coats, and eye protection.

Before you start, clean bench surfaces and pipettors with ethanol.

Plate Setup and Incubation

1d 0h 30m

1 Read the procedure listed below. Discuss with your lab partner the key steps to program the liquid handler to successfully complete this task. Pay attention to the reagents, tube format, and equipment we have. Remember the goal: **reproducibly** testing compounds (with replicates) and including negative and positive controls.

2

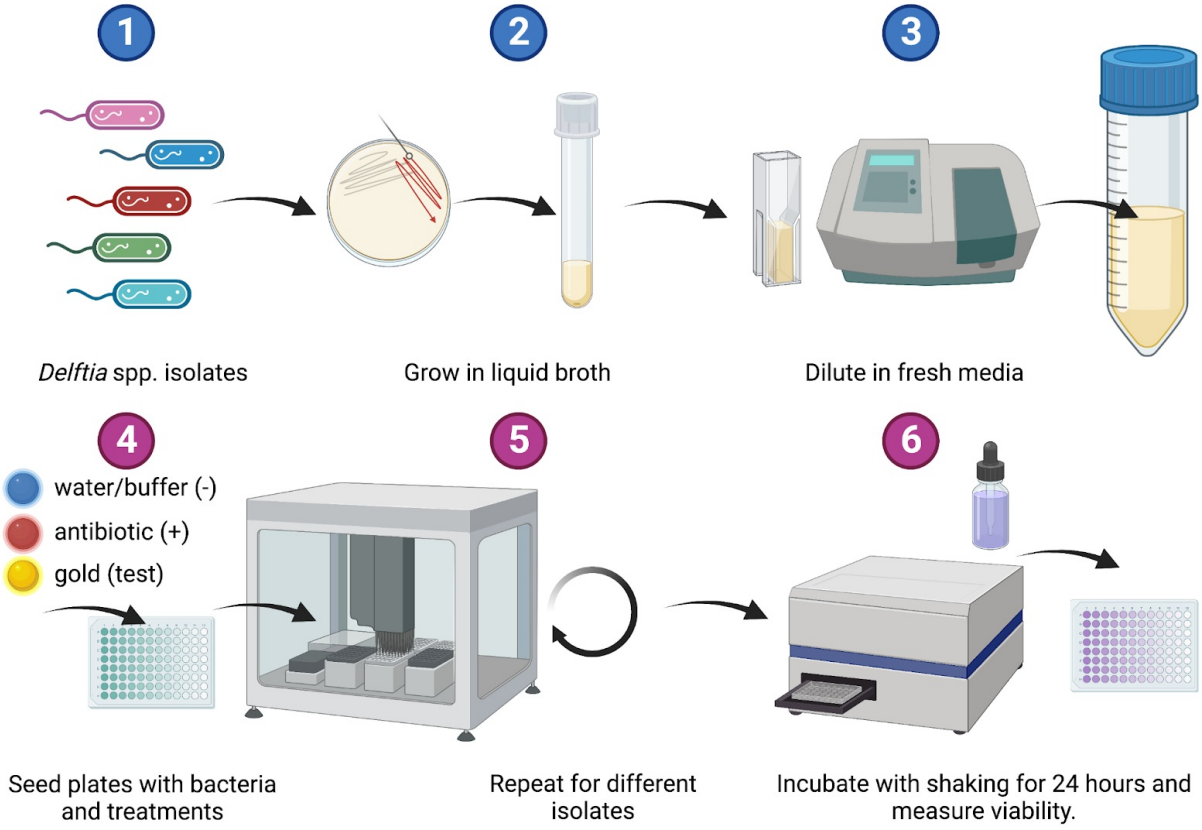

Figure 1. Schematic of procedure to use liquid handler to seed plates with bacteria and treatments. A set of *Delftia* spp. Bacterial isolates were grown on Tryptic Soy Agar (TSA) and then liquid broth (Tryptic Soy Broth) before diluting to a specific OD_{600}. The eqMotion 5075 liquid handler was used to seed plates with bacteria and treatments: water/buffer control (negative control), antibiotic (positive control), and gold chloride (test compound). Plates were incubated with shaking in a LogPhase 600 for 24 hours with OD_{600} readings obtained every 10 min. After 24 hours, Invitrogen PrestoBlue viability stain was added to all wells to quantify viability. Each condition was tested in triplicate wells for calculations of inhibitory concentrations. Created with BioRender.com

As a group, we will use a common script and the liquid handler to prepare sets of plates for analyses. Each bay will set up one plate.

3

Grow Overnight cultures of *Delftia* spp. in 2.5 mL of TSB, shaking at 300 rpm and incubating at 30 °C

4 For each strain, subculture the bacteria to approximately 5 x 10^5 CFU in 25 mL of fresh TSB by performing the following:

4.1 Measure the OD_{600} of the overnight culture (1:10 dilution in TSB);

4.2 Plug this number into the formula at the bottom of the page and multiply by 10 to ensure we have enough bacteria in each well.

4.3 Take the calculated amount (µL) from the overnight culture and add to 25 mL of fresh tryptic soy broth (TSB); mix well.

5 Add 100 µL of diluted bacterial suspension into each well of rows 2-12.

6 Add 194.88 µL of subcultured bacterial suspension into each well of row 1.

7 Add gold chloride (5.12 µL from a stock solution) to wells B1, C1, and D1.

8 Add antibiotic solution (5.12 µL from a stock solution) to E1, F1, and G1.

9 Add water (5.12 µL of water) to A1 and H1 (to ensure the diluent is not toxic to the bacteria)

10 Thoroughly mix the wells in column 1 and remove 100 µL and add to the wells in column 2.

11 Thoroughly mix the wells in column 2 and remove 100 µL and add to the wells in column 3.

12 Continue the serial dilution down the 96-well plate and discard the last 100 µL from column 12 (Note: There should be 100 µL in each well now).

13 Incubate at 30 °C for 24:00:00 with 300 rpm LogPhase600 setting in the LogPhase 600 plate reader. Take A600 readings every 10 min. 1d

14 Inspect the 96-well plate for growth. The **MIC (Minimum Inhibitory Concentration)** is the first concentration at which no growth is observed. (Note: there should be growth in rows A and H).

15 Read OD_{600} and add 10 µL of PrestoBlue to each well.

16 Incubate at 30 °C for 00:30:00 . Read OD_{570} and OD_{600} for normalization. Review the information provided in the PrestoBlue resource. 30m

17 Note the concentration at which each compound inhibits the growth of the bacteria. Record data in your electronic lab notebook.

Note

[We will complete steps 14-16 for you and send you the data.]

18 Plate Layout

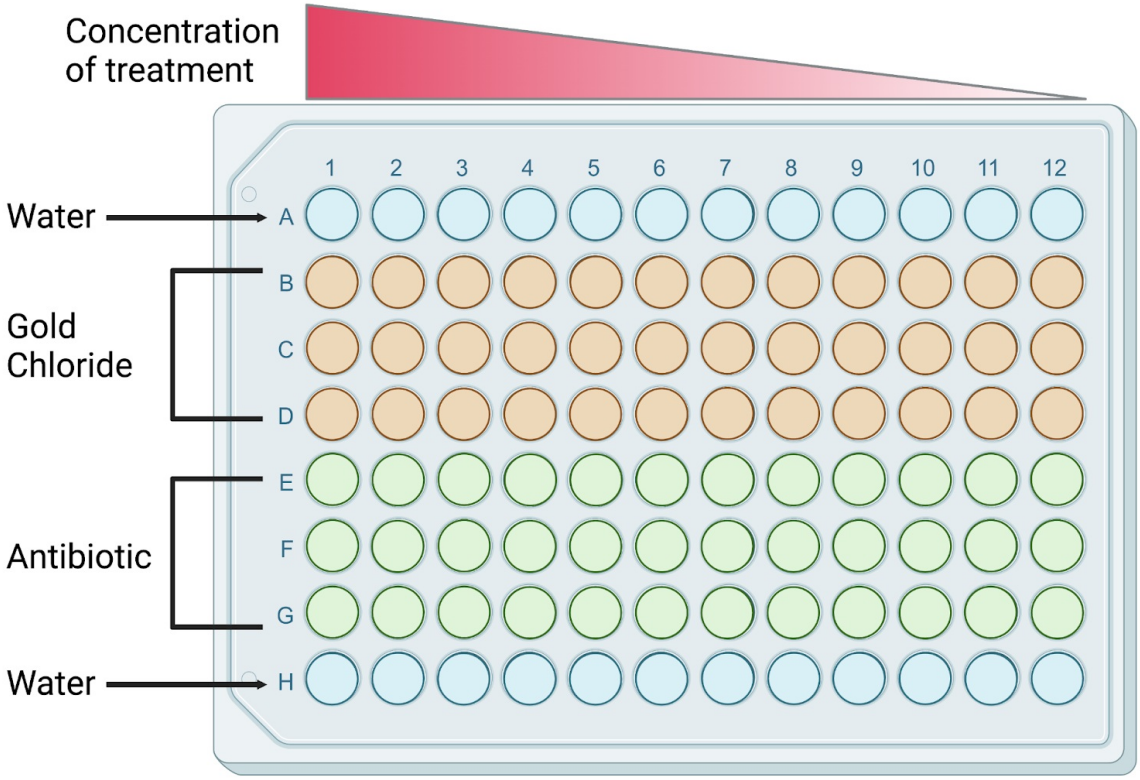

Figure 2. Plate layout for growth inhibition curves. Each row of the 96-well plate will be seeded with bacteria treated with water, antibiotic, or gold chloride. The columns of the plate will be used to dilute the concentration of the treatment to determine inhibitory concentrations. Created with BioRender.com

Note

Formula: µL of overnight culture to take = $(100/OD_{600})$*0.0005***25 ml * 10**

19

Note

Analyses

1. **Explain** how the viability stain PrestoBlue works.
2. Calculate each stain's mean and CV normalized PrestoBlue absorbance.
3. Calculate the **percent inhibition** produced by each treatment with respect to the water control.
4. Calculate the **% inhibition** produced by each compound with respect to the water controls.
5. Calculate **IC_{50}** for each treatment you tested.
6. **Summarize** your results in a table that includes: IC_{50}, treatment, and observations for all plates.

Formula

$$\mu L \text{ of overnight culture to take} = (100/OD_{600})*0.0005*\mathbf{25ml}$$