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Determination of antibiotic resistance in algal strains

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We use this protocol and it's working

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Abstract

This protocol outlines a method to assess antibiotic resistance in green algae by culturing strains in media containing varying antibiotic concentrations. Growth is monitored visually or via optical density. The assay helps identify resistant strains and evaluate the effectiveness of antibiotics in algal selection or contamination control.

Determining Optimal Geneticin and Gentamicin Concentrations:

1 Geneticin and gentamicin were diluted to concentrations of 50ug/mL, 25ug/mL, and 10ug/mL with Woods Hole/BBM media.

2 Each concentration of antibiotics was used to fill 10 wells in a honeycomb plate with 395uL of media.

3 Media without antibiotics was used to fill another 10 wells with 395uL of media.

4 5uL of algae culture was added to each well containing media.

5 The algae were left to grow at 21 C and exposed to 150uE of full spectrum light (16/8h). Results were recorded after 1 week.

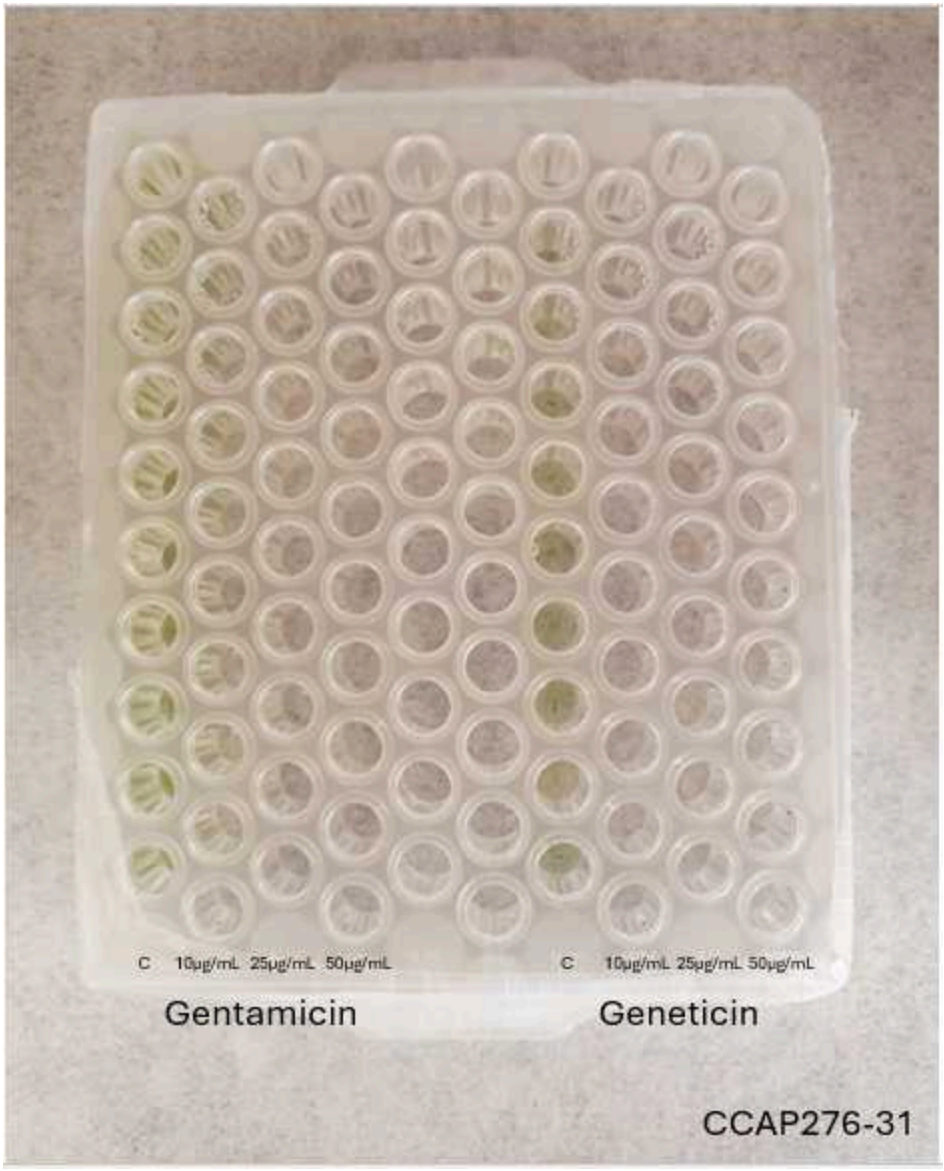

Figure 1. Honeycomb plate of CCAP276-31 after one week of growth in different concentrations of gentamicin and geneticin. The control wells, labeled C, contained no antibiotics. There is growth visible in both of the control columns and no growth in any of the antibiotic wells.

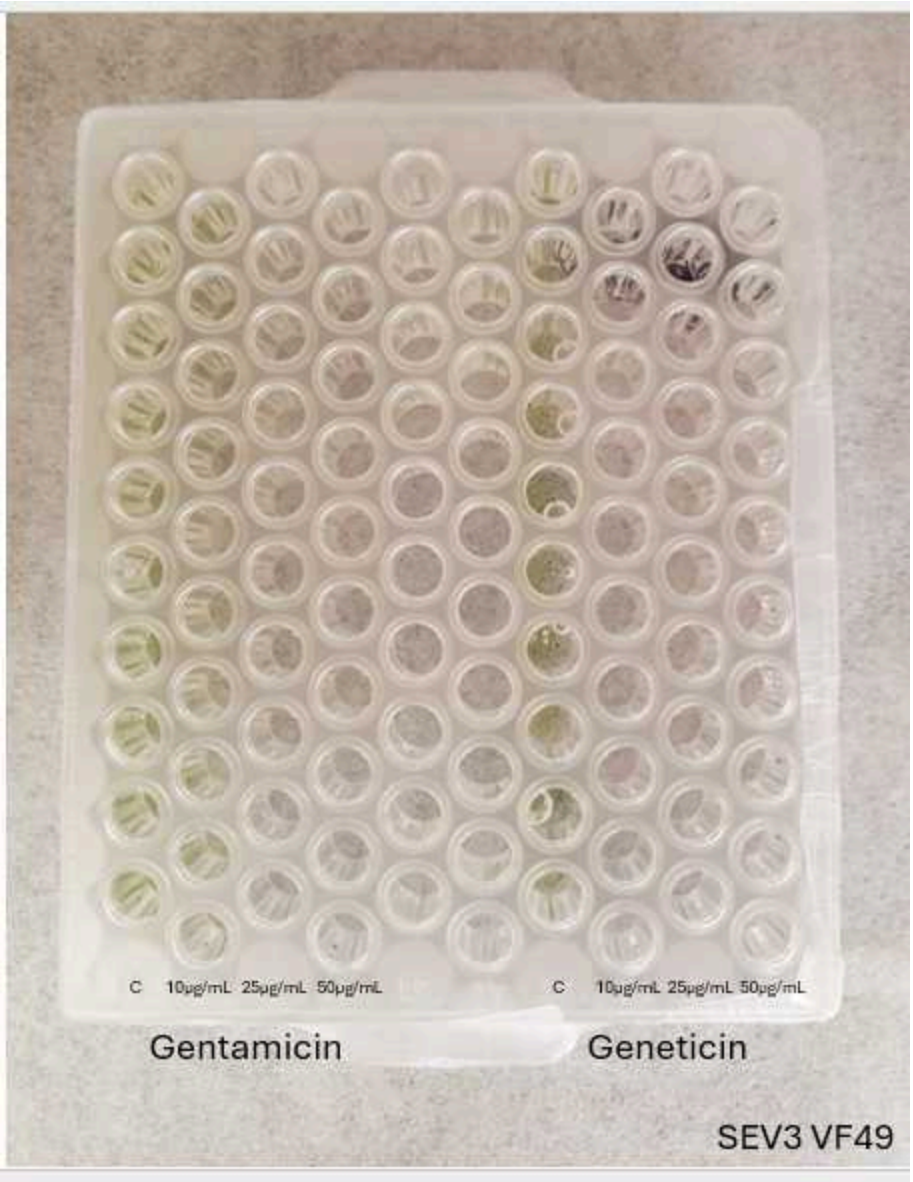

Figure 2. Honeycomb plate of SEV3 VF49 after one week of growth in different concentrations of gentamicin and geneticin. The control wells, labeled C, contained no antibiotics. There is growth visible in all of the control wells and no visible growth in any of the antibiotic-containing wells.

Figure 3. Honeycomb plate of SNI-2 after one week of growth in varying concentrations of geneticin and gentamicin. The control wells (C) contained no antibiotics. There is visible growth in the control wells and no growth visible in any of the antibiotic-containing wells.

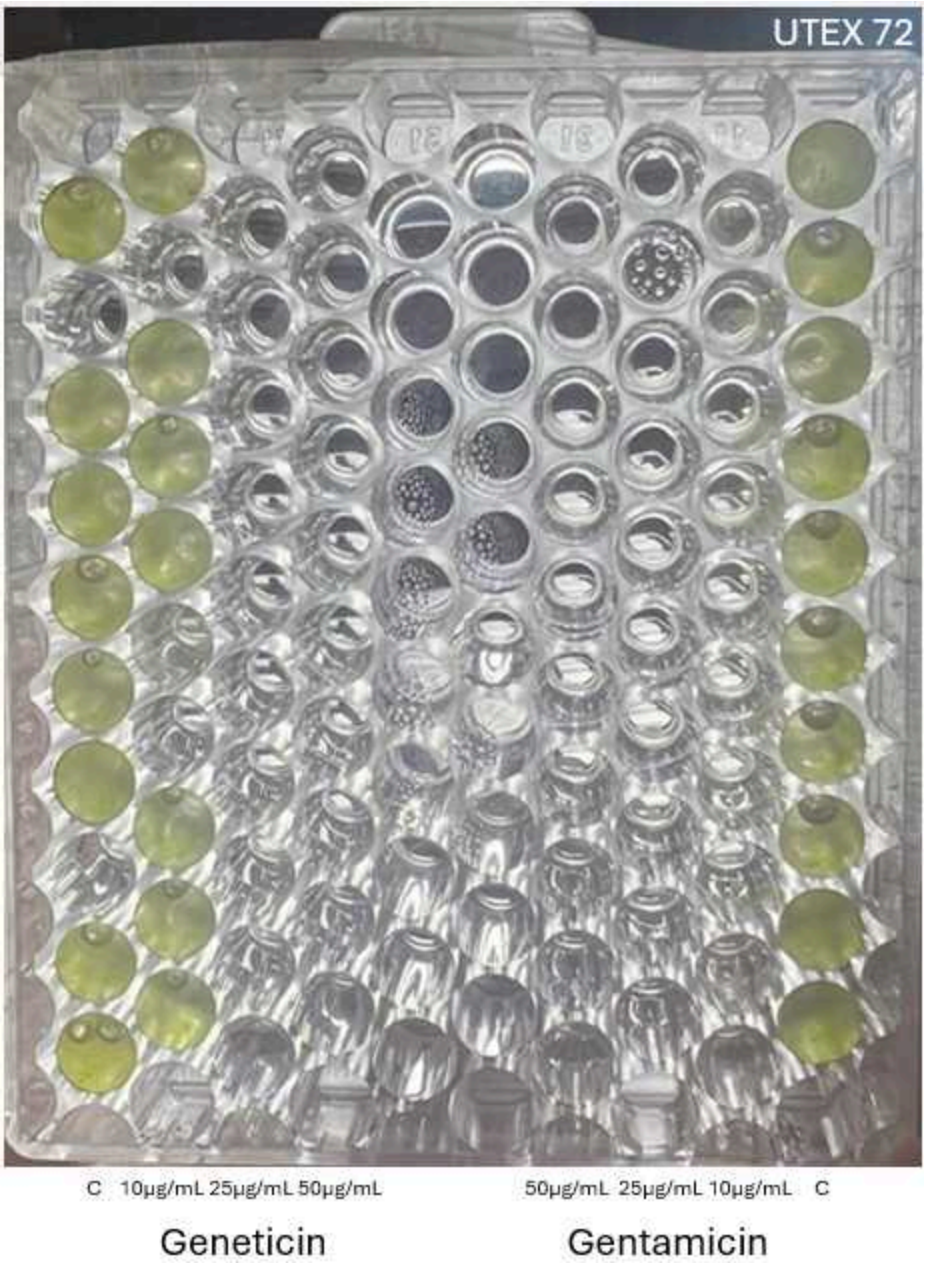

Figure 4. Honeycomb plates of UTEX 72 after one week of growth in varying concentrations of geneticin and gentamicin. The control wells (C) contained to antibiotics. There was visible growth in the control wells and the wells containing 10ug/mL geneticin.

6 Plates were allowed to continue growing for up to 6 weeks to ensure long-term growth inhibition.

Confirmation of Antibiotic Resistance on Plates

7 The results of antibiotic testing on the honeycomb plates was confirmed by plating the algae on control plates, geneticin plates, and gentamicin plates.

8 Both sets of antibiotic plates were made with 25ug/mL of the respective antibiotic.

9 The algae was plated at three different concentrations, 0.1x, 0.05x, and 0.025x.

10 The 0.1x was made with 90uL of media and 10ul of algae culture, the 0.05x was made with 95uL of media and 5uL of algae culture, and the 0.025x was made with 97.5uL of media and 2.5uL of algae culture.

11 Control, geneticin, and gentamicin plates were made with each of the three concentrations of algae for each of the four species of algae being tested.

12 The algae was left to grow under 150uE of full spectrum light (16/8h) at 21 C while being observed for growth.

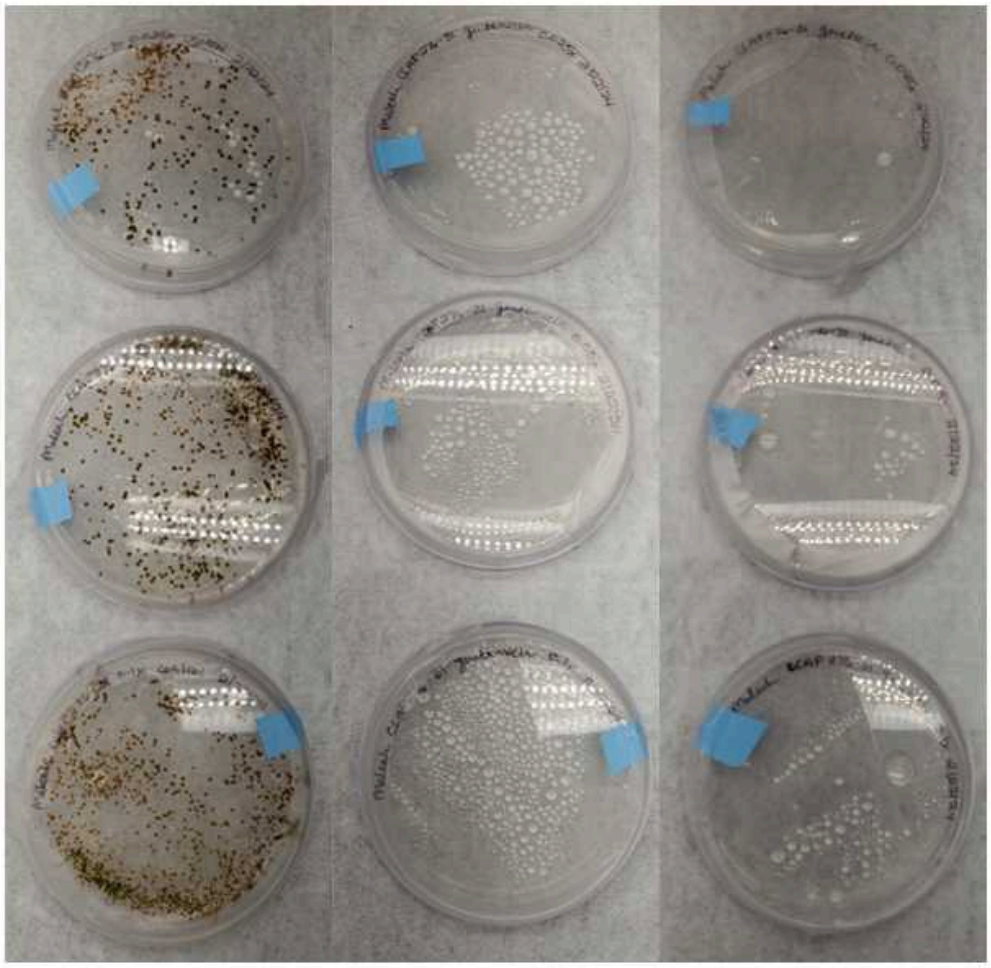

Figure 5. CCAP276-31 plated on 0.8% agar plates with no antibiotics, 25ug/mL gentamicin, and 25ug/mL geneticin. Growth is visible on all of the control plates and no growth is visible on any of the antibiotic plates.

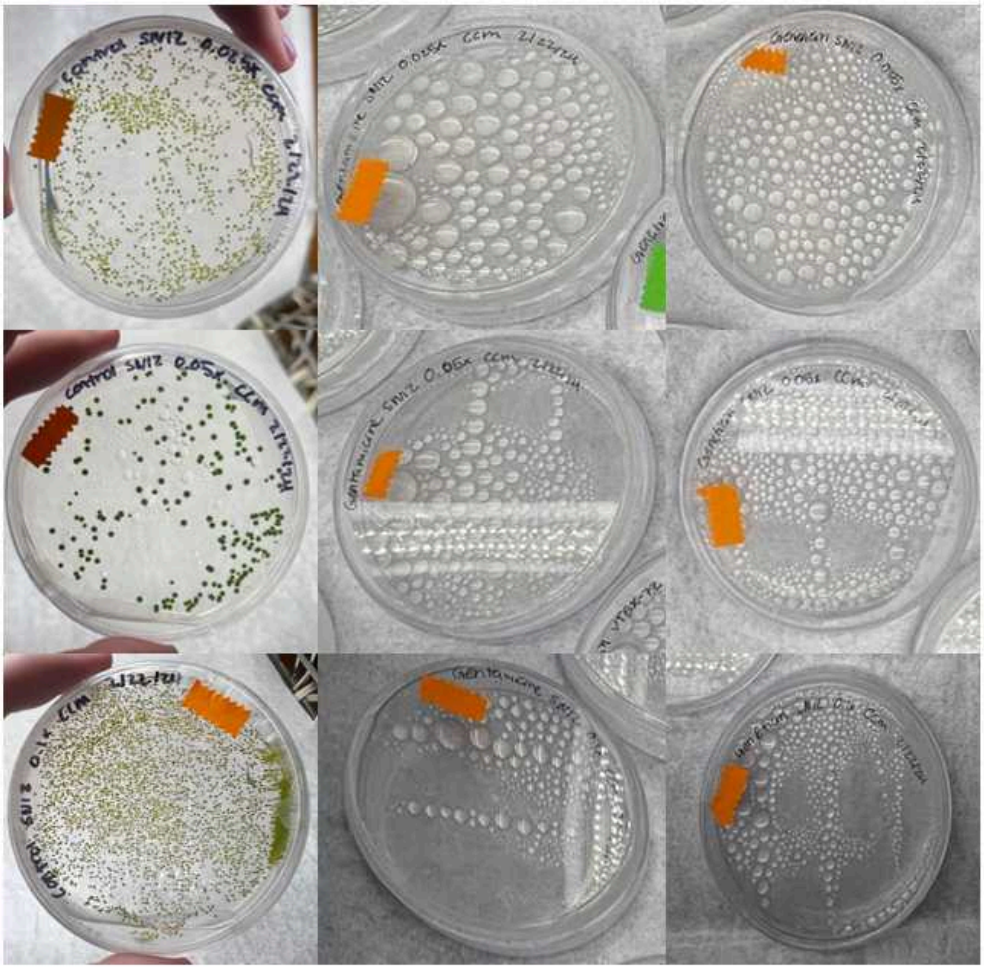

Figure 6. SNI-2 plated on 0.8% agar plates with no antibiotics, 25ug/mL gentamicin, and 25ug/mL geneticin. Growth is visible on all of the control plates and no growth is visible on any of the antibiotic plates.