**D-Amino Acid Utilization in Algae**

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**Introduction**

The D-form of amino acids is thought to be toxic to most living things, although it is known from another project in our laboratory that bacteria can grow on D-amino acids by running their racemases in reverse. This work was motivated by the recent discoveries of D-amino acids in soils and waters, which are derived primarily from cell wall (peptidoglycan) remnants of bacteria. Algæ, which are composed exclusively of L-amino acids, may help mop up the toxic D-amino acid from our environment so that we are not exposed. However, racemases are not known to be present in algæ. So the question arose as to whether algæ can detoxify D-amino acids or if they rely on associated bacteria to remove D-amino acids for them.

**Objective**

This study was conducted to determine if algæ contain racemases that would detoxify D-amino acids, or if the presence of D-amino acids would have no effect on algal growth. By using amino acid as the main nitrogen source, this hypothesis can be tested experimentally.

**Materials and Methods**

Different algal strains (Chlorella sorokiniana and Euglena gracilis) were allowed to grow in their selective mediums before distribution into 24 mL test tubes. In Table 1, the description for BG-11 medium is listed which was used for Chlorella. For Euglena, P-49 media was used, which is found in Table 2. For each trial, 16 test tubes were used: 4 D-amino acid without NaNO₃ media, 4 L-amino acid without NaNO₃ media, 4 control with NaNO₃, and 4 control without NaNO₃ media. Each tube contained 5 mL of medium, 5µL of amino acid, and 200 µL of algæ. The tubes were allowed to grow continually, and were measured in the spectrophotometer (to determine growth of algæ) one to two times per day. A 50 µL aliquot was retrieved each day, of which 20 µL were added to a vial containing 20 µL sodium borate and 10 µL of a stock solution (4 mg o-phthalaldehyde in 300 µL methanol, 500 µL sodium borate, 15 µL NAC) to analyze in the HPLC machine (to determine amino acid derivative levels).

**Results**

**Effects of Aspartic Acid on Euglena**

![Graph showing amino acid concentration (shown in purple and red) while also monitoring the growth of algæ (shown in blue and green). The algae continue to grow in D-Asp even though the main quantity of NaNO₃ is not present in the media with the level of amino acid remaining constant, giving rise to Euglena utilizing the trace amount of NaNO₃ found in the Trace Minerals solution.]

**Effects of Leucine on Chlorella**

![Graph showing amino acid concentration (shown in purple and red) while also monitoring the growth of algæ (shown in blue and green). The increase of D-Leucine in solution while the absorbance of algæ was red (while also monitoring the growth of algæ (shown in blue and green). The algae continue to grow in D-Asp even though the main quantity of NaNO₃ is not present in the media with the level of amino acid remaining constant, giving rise to Euglena utilizing the trace amount of NaNO₃ found in the Trace Minerals solution.]

**Conclusions**

The findings gave reason to believe that certain D-amino acids can be used by algæ, given the decrease in concentration in the samples. The level of aspartic acid did not display signs of decrease even though Euglena continued to grow. This can be interpreted as this D-amino acid is not toxic on the algæ and does not inhibit growth. As noted, there is a trace amount of nitrate (NO₃⁻) in the Trace Minerals Solution, which could be the reason for growth. Other research in our lab has shown a decrease in both D- and L-aspartic acid in Scenedesmus, which poses the question of why it did not decrease in Euglena as well. In Chlorella, the presence of leucine caused a decrease in both of the amino acids while drastically affecting the growth of the algæ in the D-amino acid test tube. Inside the D-Leucine tubes, it appeared as though the algæ died leaving remnants of the cells behind as a source of D-amino acid which was released into the supernatant.

**Importance of Research**

A better understanding of relationships between bacteria and plants/animal have implications in agriculture and medicine. For example, it is true that gut bacteria are responsible for removing D-amino acids for us, then a patient who is taking antibiotics may have to be careful about what to eat, because some food, such as yogurt and cheese, contain bacteria and D-amino acids.

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