Fungal Species Cross-Reactivity in Semen Testing (Acid Phosphatase)

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Introduction

Acid phosphatase (AP) is an enzyme used in forensic biology laboratories as a preliminary indicator of seminal fluid due to its high concentration. Other substances, including fungi, contain AP, and can produce positive results in these tests. The aim of this project was to further evaluate the level of enzymatic activity in fungi. This involves modification of traditional AP tests to a spectrophotometric absorbance method. In this work, a spectrophotometric quantitative method was developed to test for enzymatic activity. The protocol was developed to detect the presence of other products visible in the UV-Vis spectrum. Samples were evaluated in the wavelength range 350-600nm using the Genesys 10UV spectrophotometer. The highest absorbance wavelength, 368nm, was used to evaluate enzymatic activity. This research evaluates a variety of different edible mushrooms in this modified AP test protocol.

Objectives

- Develop a spectrophotometric method in order to evaluate enzymatic activity
- Evaluate the enzymatic activity of fungal samples using the Genesys 10UV spectrophotometer

Materials and Methods

- AP reagents were prepared according to standard protocol.
- Each sample was cut into a 1mm x 1mm x 1mm piece from each of the 8 species tested and placed into a tube.
- 200 µL of reagent 1 was added to the tube, and after 30 seconds 200 µL of reagent 2 was added.
- After 15 seconds, 50 µL of the reagent mixture is added to a cuvette containing 950 µL of water.
- Once the AP mixture is added, the cuvette was immediately put into the Genesys 10UV Spectrophotometer.
- The Advanced A-%T-C test at 368nm and the wavelength were run on the Genesys 10UV Spectrophotometer for each of the samples tested.
- The absorbance at the target wavelength was recorded for each sample.

Results

- All samples demonstrated acid phosphatase activity.
- All samples generated the expected peak at the target wavelength on the spectrophotometer.
- No additional peaks were observed in any sample.

Results

Mushroom Type (Cap)	Absorbance @368
Oyster	0.728
Shiitake	0.789
Baby Bella	0.724
Chanterelle	0.951
Trumpet	0.640
Black Trumpet	1.153
Dried Porcini	0.942
Enoki	0.734

Table 1. Absorbance Values of Various Species of Mushroom
All species present in the table were cut from the cap. The absorbance value at 368nm was recorded. Each sample was further evaluated to ensure there were no other peaks present.

Mushroom Type	Absorbance @368
Shiitake (stem)	0.982
Shiitake (gill)	0.652
Baby Bella (stem)	0.828
Baby Bella (gill)	0.781
Dried Porcini (gill)	0.962
Dried Porcini (stem)	0.860
Enoki (stem)	0.938
Enoki (root)	0.810

Table 2. Absorbance Values of Various Locations on Four Different Mushroom Species
Samples from various locations on the mushroom were tested in order to determine whether location affected the wavelength. The absorbance value at the target wavelength (368nm) was collected. No other peaks were present on the generated scan for any sample.

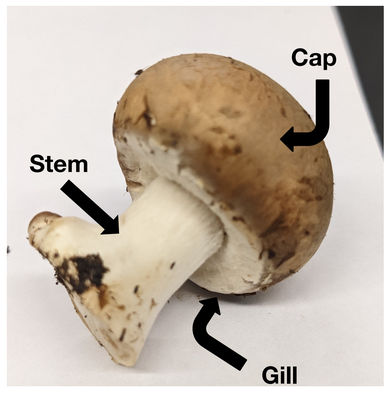

Image 1. Whole Baby Bella Mushroom
1mm by 1mm by 1mm samples were taken from the stem, gills, and cap of this species of mushroom. The generated wavelengths of these locations were then compared.

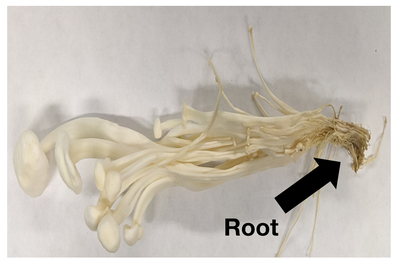

Image 2. Whole Enoki Mushroom
1mm by 1mm by 1mm samples were taken from the stem, gills, and root of this species. The generated wavelengths at the target value of each location were compared.

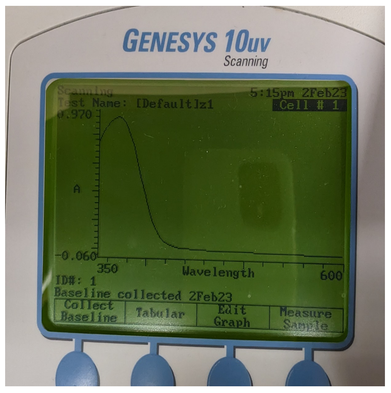

Image 3. Example of Scan on the Genesys 10UV Spectrophotometer
A scan for each sample was taken and generated a graph similar to the ima above.

Discussion and Future Work

- Future work may include: DNA extraction and human DNA quantitation, and microscopic examination of fungal samples.
- Extraction and quantitation of human DNA is especially relevant as they can potentially exclude a human AP source, including human semen. This would be performed using methods used in DNA casework.

References

- Saferstein, Richard. *Forensic Science Handbook.* Second edition., Prentice Hall, 2002.
- Li, Richard. *Forensic Biology.* Second edition., Routledge, 2015.