Engaging an Undergraduate Researcher to Develop a Mushroom Tyrosinase Assay as a Summative Assessment Device for an Upper-level Biochemistry Laboratory Course

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ABSTRACT

Working with an undergraduate enrolled in 2-credits of independent research a mushroom tyrosinase enzyme assay was researched, piloted, and optimized during the fall 2021 semester. A version of this assay was then used to write and administer a summative assessment (a lab practical) in three sections of a 6-credit capstone laboratory course for biochemistry majors. The practical evaluated students' ability to measure enzyme reaction rates, make accurate dilutions, apply knowledge of Michaelis-Menten kinetics to determine the K_m and the V_{max} of an enzyme of which they had little or no prior knowledge, and finally to gauge their ability to determine the inhibition constant (K_i) of a provided inhibitor (kojic acid).

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INTRODUCTION

Generating new course content in a large lab courses with multiple sections is a challenge, especially in courses that have been taught for many years. Providing search parameters to an independent undergraduate researcher and suggesting journals to begin looking for feasible published protocols led to the implementation of an assay that piqued the interest of the student and lab instructors while also being useful as a tool for biochemistry laboratory assessment.

While researching and optimizing this assay the undergraduate researcher gained hands-on experience troubleshooting, preparing stock buffer solutions, realizing the limitations of certain substrate solubility, and observing the time constraints of an assay in which measurable products autocatalyze to form products that are not measurable by a spectrophotometer. The development of this assay and practical exam were both extremely student-centered as the student gained practice judging the quality and feasibility of assays by searching and reading biochemistry and molecular biology education literature and communicating both via email and in-person with corresponding authors.

Pictured is 50 grams *Agaricus bisporus* about to be blended in 10 mM phosphate buffer.

METHODS

Students were provided with the following prompt. Email Kristopher@vt.edu for a full copy of the practical exam.

"Your assignment is to determine the kinetic constants for the enzyme tyrosinase within a crude extract prepared from *Agaricus bisporus*, commonly known as white button mushrooms. Mushroom tyrosinase catalyzes the oxidation of certain phenolic compounds. L-3,4 dihydroxyphenylalanine (L-DOPA) is a substrate for the tyrosinase enzyme.

L-DOPA → quinones* → dopachrome → eumelanin

*Quinone intermediates rapidly autocatalyze to dopachrome in a fraction of a second. Dopachrome (red-colored) forms and is stable for approximately 3 minutes before autocatalyzing further to eumelanin and other melanin precursor molecules (dark brown/black-colored).

You will assay tyrosinase activity by measuring dopachrome production. Dopachrome formation will be measured directly by using a continuous enzymatic assay. Dopachrome has an extinction coefficient of 3.60 mM-1 cm-1 at 475 nm.2 One unit of tyrosinase activity is defined as the amount of enzyme that catalyzes the oxidation of 1 µmol of L-DOPA per minute at room temperature. We will assay tyrosinase activity at pH 6.5."

RESULTS, CONCLUSIONS, & FUTURE STUDIES

Table 1. Example Data from the Mushroom Tyrosinase Activity Assay. Reagents, concentrations, and reaction rate values reported. All assays were performed in 1.0 mL final volume. The data indicate decreasing reaction rate with increasing kojic acid (inhibitor) concentration

Concentration of L-DOPA (mM)	12 mM L-DOPA (µL)	0.5 M Sodium Phosphate Buffer (pH 6.5)	Mushroom extract (µL)	1 mM Kojic Acid (µL)	Volume R.O. Water (µL)	Velocity (ΔA/min)		
						0 µM K.A.	10 µM K.A.	20 µM K.A.
0	0	200	200	0, 10, and 20	600	0.00	0.00	0.00
1	83	200	200	0, 10, and 20	717	0.23	0.17	0.13
3	250	200	200	0, 10, and 20	350	0.40	0.32	0.27
5	417	200	200	0, 10, and 20	183	0.57	0.50	0.41
7	583	200	200	0, 10, and 20	17	0.76	0.59	0.55

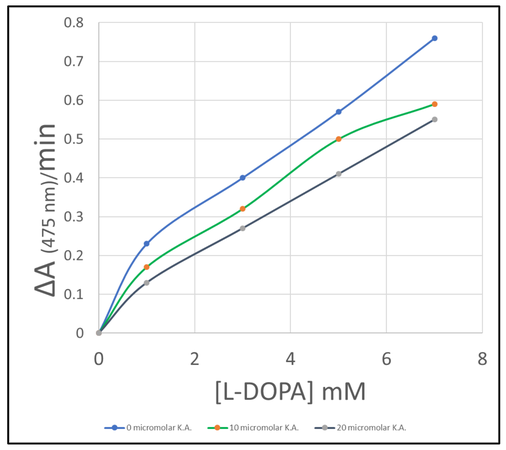

Figure 1: **Direct plot** showing the rate of reaction for L-DOPA forming dopachrome with 0, 10, and 20 µM Kojic Acid included in the assay described in table 1

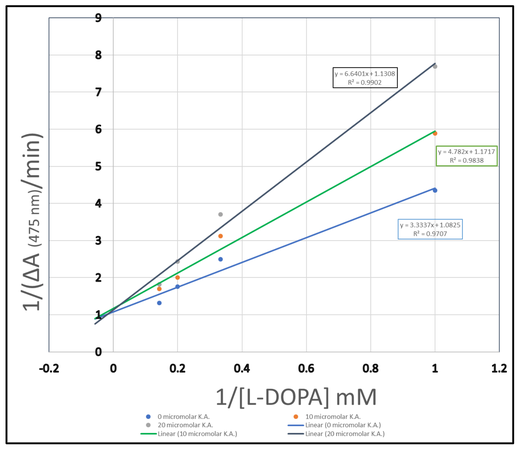

Figure 2: Lineweaver-Burk plot for the reaction of L-DOPA reacting to form dopachrome with 0, 10, and 20 µM Kojic Acid. From the plot with no inhibitor K_m was calculated to be 3.17 mM. Vmax was determined to be 0.94 ΔA/min.

Students were able to demonstrate competence measuring enzyme activity, optimizing substrate concentration, and determining kinetic constants K_m, V_{max} and K_i during this lab practical. The percentage of students who were able to independently prepare the assays, collect and interpret the data, and report constants within an order of magnitude of the expected results was used to assess teaching effectiveness. Each of three lab sections reported the percentage data which was compiled to evaluate how well agreed-upon student learning objectives were met. A common issue that arose in the practical reports was the inability of students to discern what type of inhibition was occurring from their data. Many students reported competitive inhibition when a "mixed" inhibition was expected. Future directions include using tropolone instead of kojic acid as the inhibitor. Preliminary studies shows this inhibitor clearly changes both the slope and intercept of the Lineweaver-Burk plots. I would also like to use this assay in new modules for a 2000-level biochemical techniques lab and use wild mushrooms harvested form the area as the starting sample to bserve differences in tyrosinase derived from different species.

Calvatia gigantea, aka giant puffball specimen collected near my house in Blacksburg, VA

Citations and Acknowledgments

- Flurkey, W. H., & Inlow, J. K. (2017). Use of mushroom tyrosinase to introduce michaelis-menten enzyme kinetics
- to biochemistry students. *Biochemistry and Molecular Biology Education*, *45*(3), 270–276.
- We would like to thank Dr. Jianyong Li for lively conversation about this assay and for kindly providing the L-DOPA substrate
- We would also like to thank Dr. Tim Larson for critical review of the lab practical and for improving the assay by suggesting we use more mushroom extract!

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