



Acid Phosphatase Specific Activity in Arabidopsis. Jayson Hughes and Larry L. Lowe. Biology, Chemistry, and Environmental Health Science Department, Benedict College, 1600 Harden Street, Columbia SC 29204

Abstract

Acid phosphatases are a family of enzymes present in many animal and plant species. In animals it is found in high concentrations in the epithelial cells of the prostate, but is also found in the cellular components of bone, spleen, kidney, liver, intestine, and blood. In plants it causes the hydrolysis of phosphate esters within the plant to assist in the energy metabolism and metabolic regulation of plant cells and plant growth. The motivation for conducting this research is to determine if Salk insertion mutations effects the specific activity of acid phosphatase in Arabidopsis plants compared to the non-insertion controls, and compare these results to overall plant growth and development phenotypes. Acid phosphatase specific activity was determined by measuring the conversion of p-nitro phenol phosphate to p-nitro phenol at 410nm using the Beckman Model DU-640 UV/VIS spectrophotometer. The average specific activity of acid phosphate of the Arabidopsis plant families ranged from below zero to 1.433 units/mg of total protein and the kale control plant was 0.013 units/mg of total protein. These results are significant to future work by establishing baseline acid phosphatase specific activity to be compared to acid phosphatase specific activity in Salk control and mutant Arabidopsis plants. This research was funded by a research grant from the National Science Foundation, Grant No. 521148 and NSF HBCU – UP HRD No. 1436222.

Keywords: Acid Phosphatase, Arabidopsis, Knockouts, Specific Activity

Introduction

EC (3.1.3.2) is a phosphatase used to free attached phosphate groups from other molecules during digestion: **Orthophosphoric monoester + H₂O → alcohol + H₃PO₄ (colorless reaction)**. Acid phosphatases are a family of enzymes present in many animal and plant species with molecular weights ranging from 18,000 to 100,000 grams per mole by gel electrophoresis. In animals it is found in high concentrations in the epithelial cells of the prostate, but is also found in the cellular components of bone, spleen, kidney, liver, intestine, and blood. In plants it causes the hydrolysis of phosphate esters within the plant to assist in the energy metabolism and metabolic regulation of plant cells and plant growth. The human serum levels of acid phosphatase are used to diagnose for prostate cancer elevated acid phosphatase levels and bone disease- elevated acid phosphatase levels. Normal serum levels: 0 – 0.8 units/Liter. Acid phosphatase enzymes are also used by soil microorganisms to access organically bound phosphate nutrients. Acid phosphatase in plants increases the intracellular level of phosphorus which plays a major role in plant growth and development. The purpose of this research is to determine the specific activity of acid phosphatase in control vs mutant Arabidopsis and compare these results to overall plant growth and development phenotypes.

Materials and Methods

Plant Acid Phosphatase Extraction:

- Whole plant leaves were homogenized for 60 seconds in 1ml of 40mM citrate buffer (pH 5.0) with a Benchmark BeadBug homogenizer.
- Whole extract was centrifuged at 14,000g for 2 minutes. The supernatant was designated as the crude enzyme extract.
- The crude enzyme extract was stored at – 20° C and used in acid phosphatase assay reactions.
- Acid phosphatase activity was determined (in duplicate) at 37°C for 30 minutes with 6.6mM PNPP in 40mM citrate buffer (pH 5.0), stop with 1ml of 100mM of NaOH and read at 410nm.
- **Units per mg of total protein** = $\Delta\text{abs at 410nm (rate)}/\text{ml of enzyme} \times (\text{df}) / 1\text{cm} \times 18.3 \times \text{TV} / \text{mg/ml of total protein}$



Figure 1a, Bedbug Homogenizer; 1b, Jayson Hughes; 1c, Beckman DU 640 UV-VIS Spectrophotometer

Results

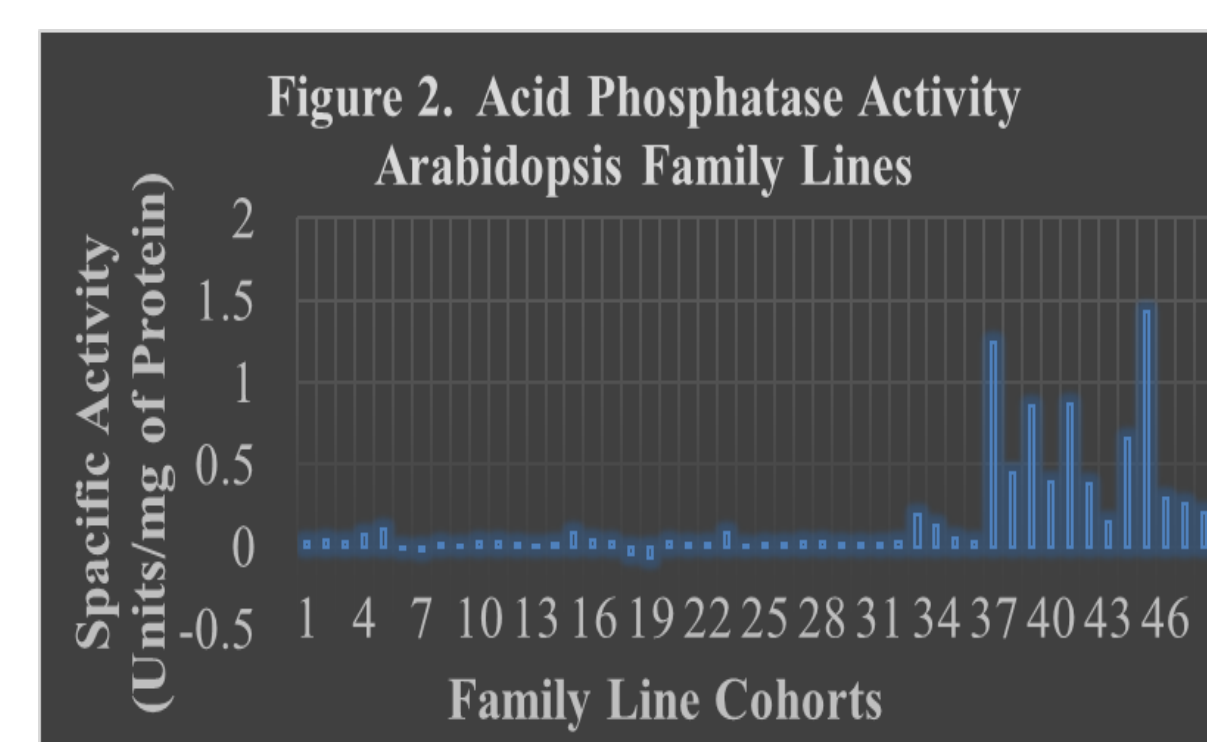


Figure 2. 1-24, Salk Chrome 4, dry; 25-36, Columbia Chrome 4, dry; 37-48, Columbia fresh

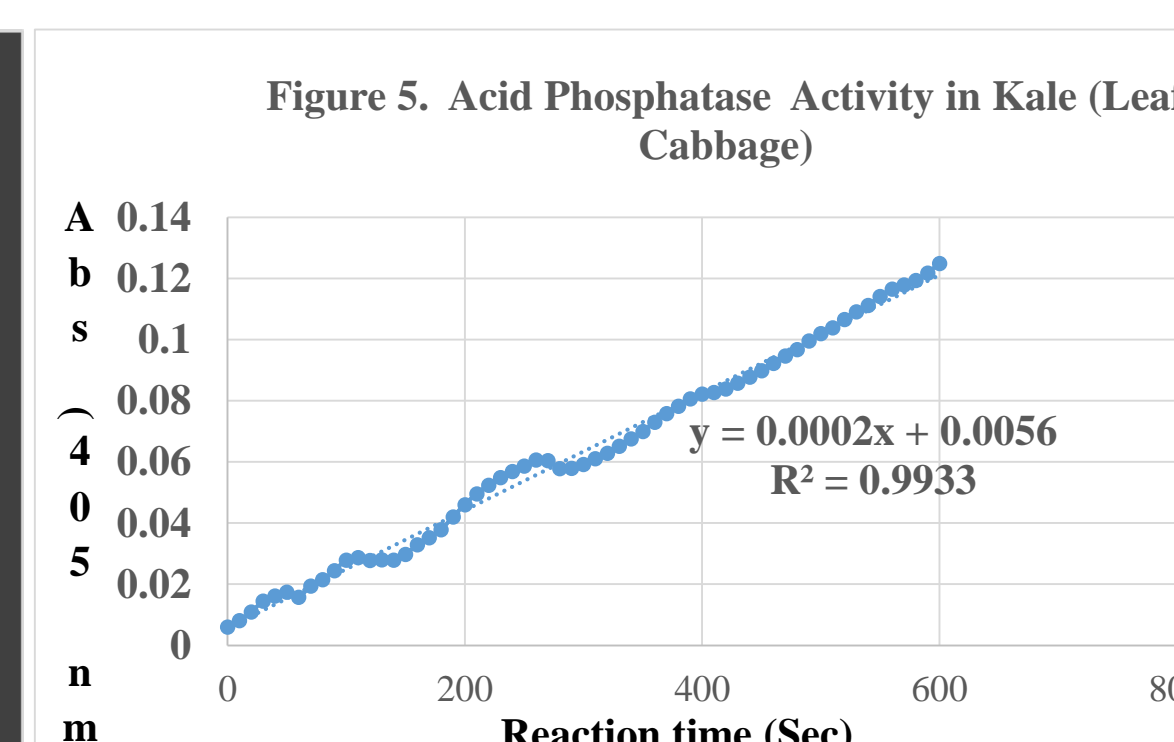


Figure 3. Acid Phosphatase Activity in Kale (external control plant)

Conclusion

- Acid phosphatase specific activity in Sigma control was **0.492 units/mg of enzyme**
- Acid phosphatase specific activity in kale (leaf cabbage: *Brassica oleracea*) was 0.113 units/mg
- Published Sigma control acid phosphatase specific activity range from 0.15 units/mg to 0.25 units/mg
- The average specific activity of acid phosphate of the Arabidopsis plant families ranged from below zero to 1.433 units/mg of total protein
- Various plant acid phosphatase specific activity in literature range from 0.1 units/mg to 2.0 units/mg

Literature Cited

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