Valorisation of an Effluent Stream: Making High Value Anti-Cancer Compounds from Daffodil Waste

Introduction and Background

Asparaginase is a bacterial enzyme used in the medicinal and food industries. Asparaginase catalyses the conversion of L-asparagine to aspartic acid and ammonia, depleting serum L-asparagine and starving the leukemic cells of the L-asparagine necessary for DNA, RNA and protein synthesis, leading to cell death [1]. It is currently industrially produced from enzymes for use in the treatment of acute lymphoblastic leukaemia (ALL), a cancer that contributes to 25% of cancer diagnosis in children <15 years old [2]. However, production from prokaryotic cells is often toxic and contains L-glutaminase and urease residues, leading to side effects during ALL treatment [3]. This project assesses the viability of using daffodils (*Narcissus*) as an alternative source of asparaginase for the treatment of ALL. The daffodil crop sampled is by Agroceutical Products, a company farming daffodils for the extraction of galanthamine as Alzheimer's treatment; success in this project will provide a route to valorise the waste stream of their process.

Procedure

Extraction

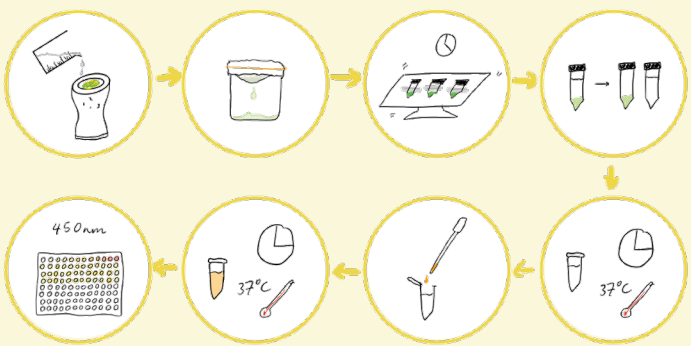
Crude daffodil samples were blended in a coffee grinder and mixed with extraction buffer - 20mM Tris-HCl (pH 8.0) containing 10% (v/v) glycerol, 50 mM potassium chloride (KCl),12.5 mM 2-mercaptoethanol and 1 mM PMSF (halt protease inhibitor cocktail). Sample was left to filter through muslin cloth overnight.

Purification

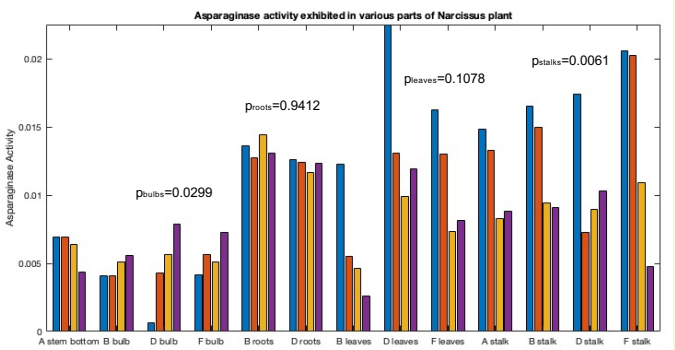
Liquid sample was applied to DE52 matrix, previously equilibrated with 0.02M potassium phosphate. First pass was collected. Enzymes were eluted by 0.1M, 0.2M and 0.3M KCl. Elution buffer was added and sample was incubated for 20 minutes at room temperature. Sample was centrifuged at 1500g for 5 minutes and supernatant was collected.

Asparaginase Activity Determination

50μL of each sample was added to 50μL of 20mM L-asparagine and 100μL of 0.02M potassium phosphate. Samples were incubated at 37°C for 15 minutes. 350μL of 1.5M trichloroacetic acid was added to stop the reaction. 350μL of Nessler's reagent was added to each sample. Samples were incubated at 37°C for 15 minutes. 200μL of each sample was placed in a black-sided 96 well plate and optical density was read at 450nm. Activity was determined by comparing optical density to 0-0.02μM ammonium sulphate standard curve. 1 unit asparaginase activity = amount of enzyme that liberates 1 μmol of ammonia per minute at 37°C.



Results



Asparaginase activity per gram of sample determined in *Narcissus* samples after purification by DE52 matrix (equilibrated with 0.02M potassium phosphate) and activity determination by Nessler assay. Data are presented as mean of triplicate samples for each individual sample. Significance was tested using a one-sided ANOVA. Data from first pass, first elution, second elution and third elution are shown in the blue, red, yellow and purple bars respectively.

Discussion

Conclusions

- **Roots** exhibited the highest average activity across all elution stages. However, this is not a **sustainable** farming technique since harvesting the roots would be fatal to the plant.
- Some **leaf** and **stalk** samples exhibited high activity and harvesting of these sections is less fatal to the life of the plant.
- Bulbs and stem bottom exhibited the **lowest** activity. This is in **favour** of Agroceutical Products' method of harvesting plant tops since the bulbs can be left in the ground to regenerate the plant without wasting an asparaginase-rich source
- The asparaginase activity recorded during the **first pass** (blue bar) of most samples is very high. This implies that the DE52 matrix is not an ideal choice for determining asparaginase activity from daffodils.

Areas for Improvement

- Mainly time related due to COVID-19 restrictions on laboratory time
- Repeats of more samples from stem bottom and bulb samples since these were tested first in the laboratory when the procedure was not very familiar
- Use alternative matrix to DE52 to ensure asparaginase activity levels detected in the first pass are lower
- Test asparaginase sample for L-glutaminase activity

[1] John C. Wriston. [79] Asparaginase. In *Methods in Enzymology*, volume 113 of Glutamate, Glutamine,Glutathione, and Related Compounds, pages 608–618, 01/1985

[2] Cancer Research UK, https://www.cancerresearchuk.org/aboutcancer/acute-lymphoblasticleukaemiaall/about, accessed 21/04/21

[3] Rachel A. Egler, Sanjay P. Ahuja, and Yousif Matloub. L-asparaginase in the treatment of patients with acute lymphoblastic leukemia. *Journal of Pharmacology & Pharmacotherapeutics*, 7(2):62–71, 2016

References

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