Metabolites in buckwheat cultivars and their anti-microbial properties

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INTRODUCTION

Buckwheat is an important grain in Asia and Europe. It is noted for its nutritional use and has applications in East Asian medicines. Based on its merit as a medicinal plant, it is hoped that some compound with anti-microbial properties may be discovered. Studies using soybeans, have shown significant variation in the metabolic compounds present. Previous studies have demonstrated that many plants have metabolites with anti-microbial properties, and buckwheat remains a prime candidate due to its current usage in medicine, affordability, and relative growing ease. As such, the project hopes to identify anti-microbial metabolites present in buckwheat and also address the metabolic variation among cultivars. In this project, metabolites from buckwheat were extracted and applied to gram-positive and gram-negative bacteria to check for a circle of inhibition.

MATERIALS & METHODS

Materials

- Metabolite Extraction Kit (plant extraction buffer, protease inhibitor cocktail 200X, homogenization pestles, homogenization tubes)
- Organic Buckwheat (Bob's Red Mill)
- Gram-positive/gram negative bacteria culture (E-coli/Bacilius)
- LB agar
- Micropipette
- Hole-puncher
- Cardstock
- Tweezers
- dH2O

Procedure

1.Use the metabolite extraction kit to extract from the buckwheat following the manufacturers' instructions
2.Plates
   1. Hole-punch the cardstock and then
   2. Autoclave the LB agar until fully dissolved
   3. Pour the agar into the petri dishes and place the hole punched card stock onto the agar with a pair of tweezers (1 for each corner of the petri-dish)
   4. Leave plates at room temperature until cool
   5. Place plates into fridge until use
   6. When done, use tweezers to peel off the paper disks
3.Pipette 10uL of extract, 10 uL of ampicillin dilution, and 10 uL of dH2O in one well each
4.Spread the 24 hr bacteria culture onto the disk
5.Place the plate into the incubator for the bacteria to grow
6.Remove plates from the incubator and chill
7.Take pictures of the plates from above with a reference length
8.Using ImageJ measure the zone of inhibition

RESULTS

Bacilius

dH2O	0 cm
Ampicillin	1 cm
Ground Buckwheat	0 cm
Whole Buckwheat	0 cm

E. Coli

dH2O	0 cm
Ampicillin	.8 cm
Ground Buckwheat	0 cm
Whole Buckwheat	0 cm

SUMMARY / CONCLUSIONS

- Results were inconclusive and needed further analysis since the antimicrobial compounds may not have been in a high enough concentration
- It was confirmed that there were no procedural errors because there was no zone of inhibition for dH2O
- The experiment required a greater number of trials due to the large variation between buckwheat and the fungus commonly present in buckwheat
- In addition, the concentration of ampicillin was an unknown concern
- With the metabolite extraction process it is possible that we created new compounds not previously present in buckwheat

FUTURE RESEARCH

- The sample size for buckwheat needs to be significantly larger due to variation beyond our control during the growing and processing
- Another process might be to analyze the chemical components of buckwheat and determine individually whether there is enough anti-microbial compound
- The goal is to eventually identify the compounds responsible for such antimicrobial activity, however this has enormous variance and it may not be possible to identify the specific metabolite
- Many compounds work in tandem, individually they may have no effect, but together they may combine to create the desired anti-microbial effect
- HPLC analysis would be another tool to identify the chemical compounds present within buckwheat
- In short, many more trials and analysis are needed to overcome the variation given the nature of the product
- By using different variations, it is hoped that one of the differences between the variations will be the key to discovering the right metabolite through cross comparison

ACKNOWLEDGEMENTS / REFERENCES

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