Bacterial Metabolism of Glucosinolates from Brassica: Association with Isothiocyanate Production

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PRECS Phenotypic Plasticity Research Experience for Community College Students

What are glucosinolates?

- Glucosinolates (GSLs) are plant secondary metabolites with nutritional impacts[1].
- Brassica vegetables, such as kale and broccoli, are rich in GSLs[2].
- GSLs are biologically inactive, however their metabolic products vary in bioactivity[3].
- There are multiple microbial metabolic pathways for GSLs, producing a variety of products.

Products of Metabolized GSLs

- Isothiocyannates (ITCs)- believed to be the most bioactive and desirable metabolite[3].
- Amines .
- Desulfo-GSLs
- Nitriles

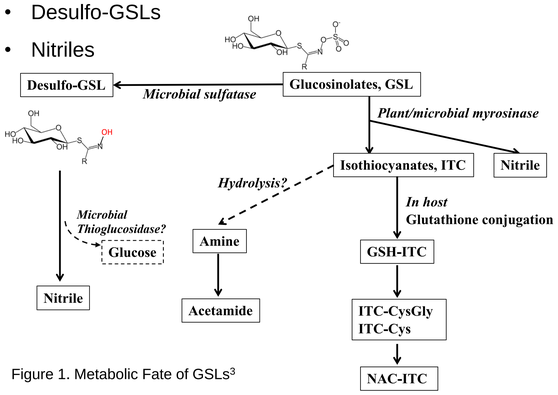

Figure 1. Metabolic Fate of GSLs[3]

What are ITCs and what do they have to do with bacteria metabolism?

- ITCs can have anti-carcinogenic, anti-inflammatory, oxidation regulation, neuroprotection, and anti-obesity properties[2:3].
- GSL conversion to ITC by the gut microbiota ranges from about 1% to over 40%[2].
- The GSL to ITC conversion pathway is extensively researched, but there is little research on alternative metabolic pathways.
- Eating brassica vegetables with GSL metabolizing bacteria on the surface could potentially change the gut microbiome.
- If bacteria utilize the alternative metabolic pathways that result in the production of desulfo-GSLs or nitriles, this could provide some explanation as to why there is a high variance in human gut microbial GSL to ITC conversion.

Study Focus:

To explore the alternative metabolic pathways of GSLs in bacteria, present on the surface of brassica vegetables.

Hypothesis:

The alternative pathways used to metabolize GSLs in bacteria located on the surface of brassica vegetables, may contribute to the high variance of human gut GSL conversion to ITC.

Procedure

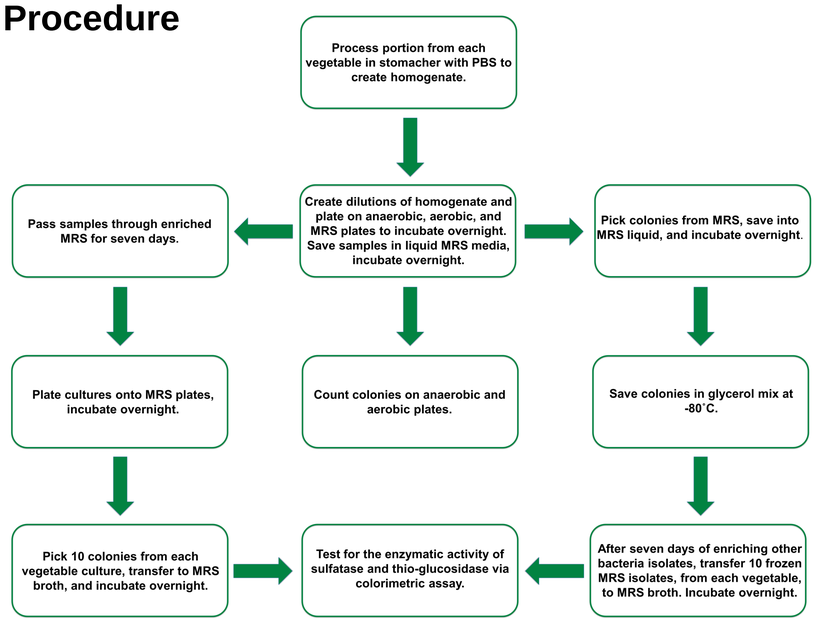

Figure 2. Procedure Flowchart

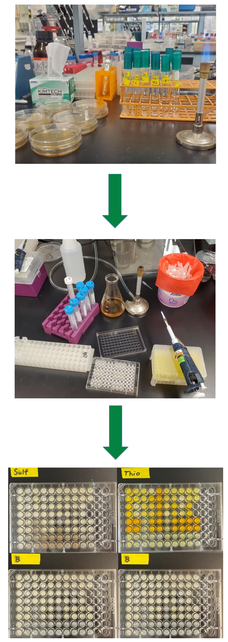

Figure 3. Procedure Visual

Results

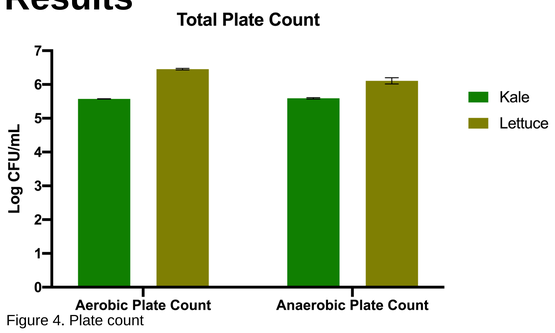

Figure 4. Plate count

- Lettuce samples have greater population growth in anaerobic and aerobic conditions in comparison with kale.

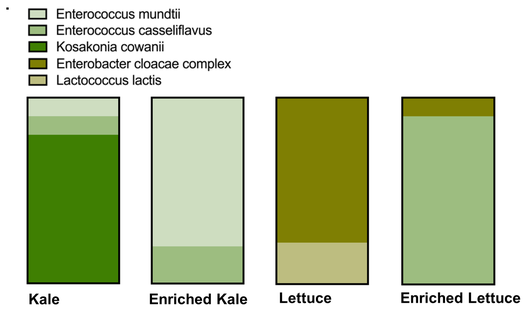

Figure 6. MALDI-TOF

Sample Group	Sulfatase +	Thio-Glucosidase +
Kale	0/10	10/10
Enriched Kale	2/10	10/10
Lettuce	0/9	9/9
Enriched Lettuce	6/10	10/10

Table 1. Enzymatic Activity

- All strains tested positive for thio-glucosidase activity.
- Eight enriched strains tested positive for sulfatase.
- Ideal next step would be to perform a cyclocondensation reaction on sulfatase + strains to confirm sulfatase, thio-glucosidase, and ITC production. Then measure the products through HPLC to evaluate.
- Samples were run through Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF) for general identification.
- This procedure compares against already identified samples available in the Veterinary Diagnostic Laboratory database, exact identification is not guaranteed.
- 80% of kale strains were potentially identified as *Kosakonia cowanii*. Identification scores suggest only the genus identification is accurate.
- To get a positive identification on these strains, the next step would be to perform 16s rRNA sequencing.

Discussion

- Strains from all samples tested positive for thio-glucosidase. Two enriched strains from kale samples and six enriched strains from lettuce samples tested positive for sulfatase activity.
- Once the sulfate group is removed, the compound can no longer be converted to the bioactive ITC[3].
- The thio-glucosidase results for the eight sulfatase+ strains are a weaker positive compared to most thio-glucosidase+ strains.
- The enriched kale and lettuce strains testing positive for both enzymatic activities were identified to be *Enterococcus casseliflavus,* with a secure genus identification and probable species identification, apart from one enriched lettuce strain. The one strain did have a probable genus identification.
- Based on current results, it can be concluded that two enriched strains from kale and six enriched strains from lettuce are utilizing the alternative GSL metabolizing pathways.
- Next step would be to confirm and quantify the sulfatase, thio-glucosidase, and ITC production activity via cyclocondensation/HPLC.
- Once these are confirmed and quantified, 16s rRNA sequencing can confirm strain identity and genes involved in the pathways can be further studied.
- To confidently accept or reject the hypothesis, performing an animal study, feeding the subjects a diet of kale and lettuce, then using similar analysis procedures on their gut microbiome would be ideal.

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3. Zhao, A.; Jeffery, E.H.; Miller, M.J. Is Bitterness Only a Taste? The Expanding Area of Health Benefits of Brassica Vegetables and Potential for Bitter Taste Receptors to Support Health Benefits. *Nutrients 2022, 14, 1434.* https://doi.org/10.3390/nu14071434

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