

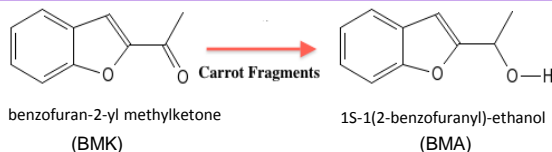
Biotransformations: Reaction Conditions, Antimicrobial Properties, and Enzyme Isolation

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ABSTRACT

Biotransformation reactions using whole cell or organisms have been extensively studied. Most of the studies have focused on producing and characterizing the enantiospecific products. Benefits of biotransformation reactions include the use of water and other environmentally friendly conditions. We decided to focus on a single reaction to determine the best reaction conditions for the biotransformation, explore possible antimicrobial activity of the product, and isolate and characterize the protein involved in catalyzing the reaction.

REACTION



METHODS

Conditions/Protein Analysis

- 8 g of carrot strips were mixed with 25 mg of ketone (1.6 X 10⁻⁴ moles) with various buffers, detergents, and methods of agitation.
- These samples were then filtered, the filtrate collected, extracted into ethyl acetate, and TLC analysis performed to verify presence of the alcohol.
- A variety of attempts were made to isolate the protein from the surface of the carrot.
- SDS-PAGE and Bradford analysis were utilized for protein analysis

Antimicrobial Studies

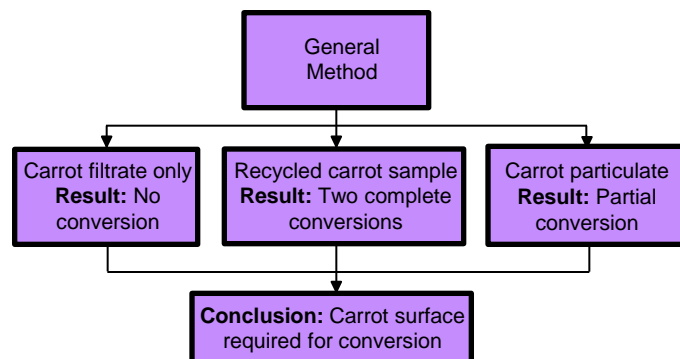
- E.Coli (BL21) and Baker's yeast were both grown in the presence of the reactant (BMK) and the product (BMA) to determine if the compounds inhibited the yeast or the bacteria's growth.

FUNDING & REFERENCES

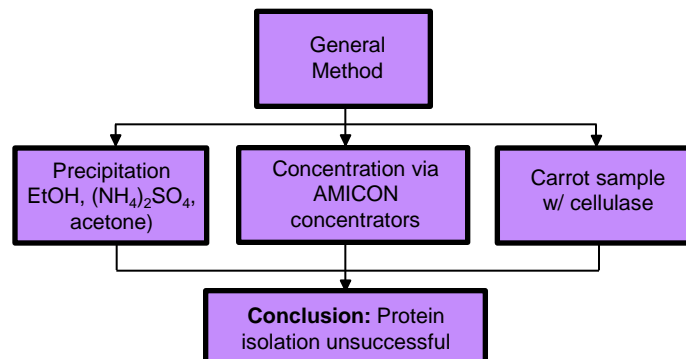
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REACTION CONDITIONS



PROTEIN ISOLATION



RESULTS

Table 1: Example of solutions of carrot samples prepared for Bradford and SDS-PAGE protein analysis.

| Lane | Buffer Used | Agitation Type | Protein Conc. (µg/ml) |
|------|------------------|----------------|-----------------------|
| 1 | Carbonate; pH 10 | Rotisserie | 350 |
| 2 | 1X TBS; pH 7 | | 400 |
| 3 | Carbonate; pH 10 | Vortexer | 589 |
| 4 | 1X TBS; pH 7 | | 439 |



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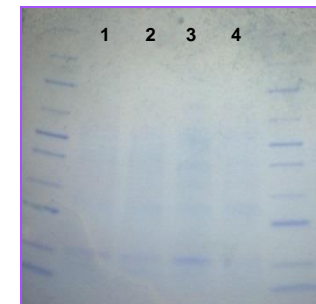


Figure 1: SDS-PAGE results for the concentrated filtrates indicates presence of protein removed from carrot surface.

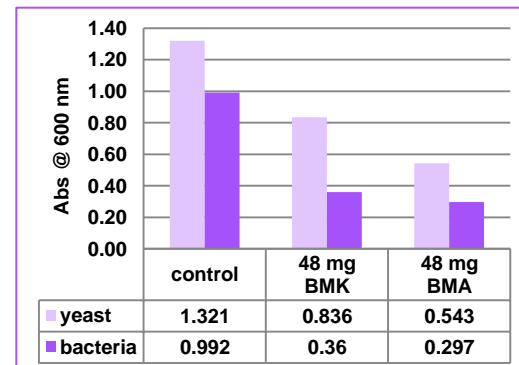


Figure 2: Inhibition of growth of yeast and bacteria.

CONCLUSION

According to the results of SDS-PAGE and Bradford analysis, the carbonate buffer with vortexer resulted in the highest concentration of protein removed from the carrot after AMICON concentration. Although the active protein was not isolated, the additional studies were carried out to gain a better understanding of the properties of the protein and its dependence on the cofactor. Future plans include working toward the isolation of the protein with a larger concentrated sample using FPLC to enhance the separation and purification of the proteins. The antimicrobial studies indicate the alcohol product has more potency at inhibiting growth in both the yeast and the bacteria.