DESIGNING, CLONING, AND TESTING GENETICALLY ENGINEERED METABOLIC PATHWAYS TO PRODUCE THERAPEUTICS IN GUT BACTERIA

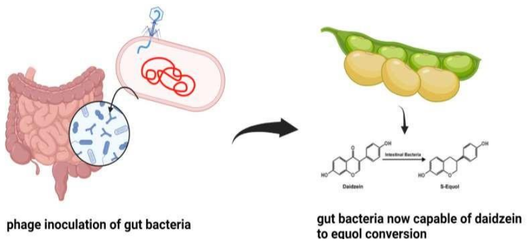
Omar Elkassih, Priyanka Shiveshwarkar, William Thomas Ingram, Suzette Martinez, Morad Marikh

Mentor: Dr. Justyn Jaworski

SUMMARY

Equol is a natural compound derived from daidzein, an isoflavone found in soybean and soy products. The presence of equol in the body has been linked to reduced risks of certain cancers and cardiovascular and hormone-dependent illnesses.

In this study, we are genetically engineering a bacteriophage, a virus that infects only *E. coli*, to carry genes necessary for isoflavone metabolization. Using genetically engineered bacteriophages, we can create a probiotic that allows consumers to receive the health benefits of soy-product-derived equol.



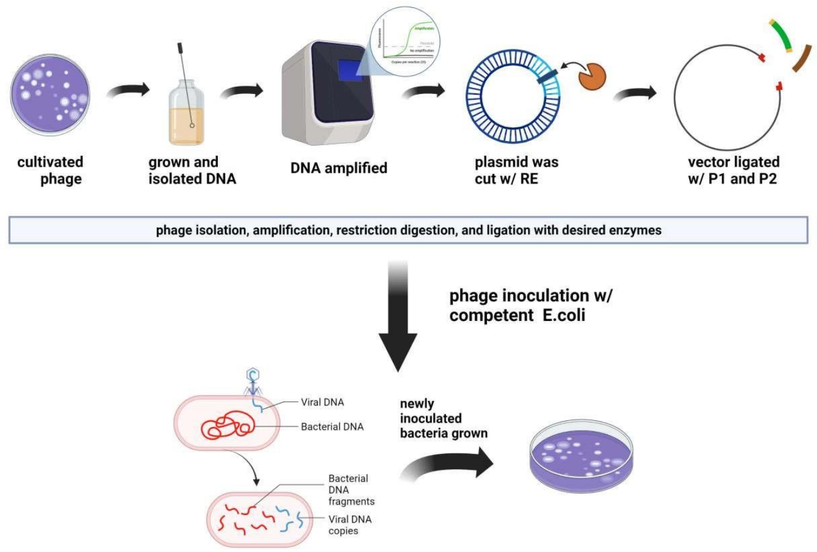
BACKGROUND

Isoflavones are a group of bioactive plant-based compounds that have received considerable attention due to their structural and functional similarities to naturally occurring hormones.

- These compounds are believed to exert their physiological effects by binding to hormone receptors, leading to downstream agonistic or antagonistic effects. [1]
- The isoflavone daidzein, found in soybeans, is of particular interest because of its conversion to equol which is associated with positive health outcomes, including a reduced risk of breast cancer and cardiovascular disease. [2]
- The enzymatic conversion of daidzein into equol is dependent on the presence of specific gut bacteria that **only a fraction of the population possesses.**

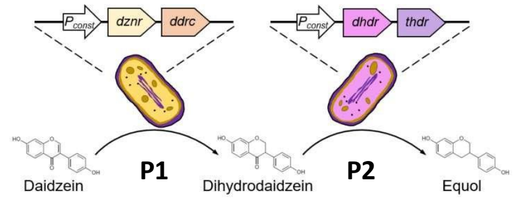
AIMS. This project is aimed to address the health disparities between individuals who can vs those who cannot metabolize isoflavones and thus to provide the health benefits of equol to all.

EXPERIMENTAL SETUP



METHODS

- **PLASMID INSERT CREATION.** The DNA plasmids (P1 and P2) were engineered through codon optimization of the desired genes from the *Lactococcus garvieae* strain to *E. coli* and purchased as gene blocks (Integrated DNA Technologies, Iowa, USA). Amplification of the genes was done through PCR using primers with BioBricks prefix and suffix and subsequent enzymatic digest using PstI, XbaI, EcoRI, and SpeI and our purified PCR product was then used as the insert for the subsequent ligation.



- **PLASMID LIGATION & TRANSFORMATION.** M13 bacteriophage-infected bacteria was cultivated and resulting colonies were isolated. After mini-prep of the phage DNA, PCR was utilized to amplify the entire plasmid and DpnI used to digest the methylated parental DNA template. The purified product was then cut using restriction enzymes (EcoRI and PstI) and then purified once again. PCR of the inserts from P1 and P2 plasmids were done to amplify our target genes as described above and then ligated into our newly constructed M13 vector. This modified phage DNA was then transformed into a competent strain of E. coli (DH5α) and grown on LB + IPTG/Xgal plates to confirm the removal of the original lacZ gene. The plasmid-containing cells capable of producing the engineered phage were then grown in LB culture for isolation by precipitation with PEG (polyethylene- glycol) and storage under refrigeration for further analysis.

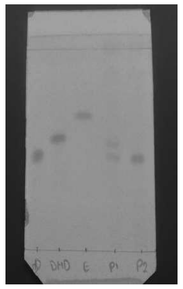
EXPERIMENTAL RESULTS

(Panel A) Thin layer chromatography (TLC) of daidzein control (lane 1), dihydrodaidzein control (lane 2), equol control (lane 3) extracted sample of cell pellet from P1 culture provided daidzein (lane 4) and extracted sample of cell pellet from P2 culture provided daidzein (lane 5), showing that the enzymes expressed in P1 carried out the conversion of daidzein to dihydrodaidzein in the isoflavone pathway but that equol will not be produced directly from daidzein with only P2 as was to be expected.

(Panel B) Thin layer chromatography (TLC) of daidzein control (lane 1), dihydrodaidzein control (lane 2), equol control (lane 3), and extracted sample of cell pellet from P2 culture provided dihydrodaidzein (lane 4), showing that the enzymes expressed in P2 carried out the conversion of dihydrodaidzein to equol in the isoflavone pathway.

(Panel C) A Double Plate Layer Assay (DPLA) shows plaques (areas of inhibited growth) of DH5α cells transformed with the engineered M13 DNA. The subsequent agarose gel shows a clear plaque picked and grown overnight, mini-prepped to extract the DNA, and then PCR'd using primers corresponding to the P1 gene. Gel electrophoresis confirms the successful integration of the P1 gene in the M13 genome.

A



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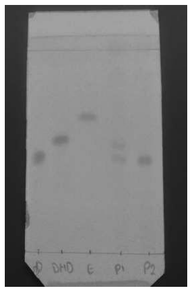

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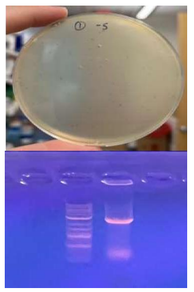
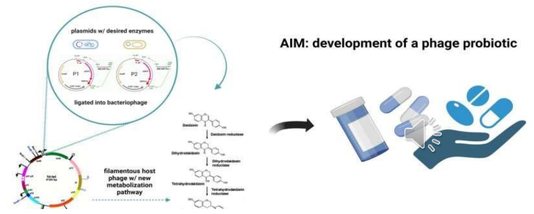


CONCLUSIONS

Our current testing has demonstrated the successful conversion of daidzein to dihydrodaidzein using the enzymes expressed in our P1 plasmid, and the conversion of dihydrodaidzein to equol using our P2 plasmid. With this, we ligated and introduced our desired genes into the M13 bacteriophage genome.

NEXT STEPS. Our next step is to conduct in vitro studies and demonstrate the use of bacteriophage transmission as a vector for providing new isoflavone conversion pathways.

LONG-TERM GOALS. Long-term future iterations of this project should focus on analyzing and identifying the specific interactions and pathways that lead to the health benefits observed in those with the equol-producing phenotype. Further insight into these interactions can give us a better idea of how the gut interacts with the rest of the body and may even allow us to replicate the beneficial effects seen by natural equol producers when consuming a daidzein-rich diet.



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1. Matthies, A., Blaut, M., & Braune, A. (2009). Isolation of a human intestinal bacterium capable of daidzein and genistein conversion. *Applied and Environmental Microbiology*, *75*(6), 1740–1744. https://doi.org/10.1128/AEM.01795-08
2. Jackson, R. L., Greiwe, J. S., & Schwen, R. J. (2011). Emerging evidence of the health benefits of S-equol, an estrogen receptor β agonist. *Nutrition Reviews*, 69(8), 432–448. https://doi.org/10.1111/j.1753-4887.2011.00400.x