



NORTHWESTERN UNIVERSITY

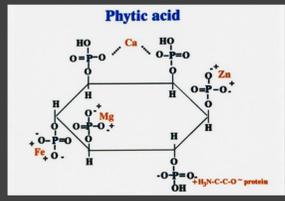
THE *Phytastic* PROBIOTIC: INCREASING THE BIOAVAILABILITY OF NUTRIENTS IN THE DIGESTIVE SYSTEM

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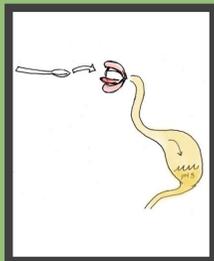
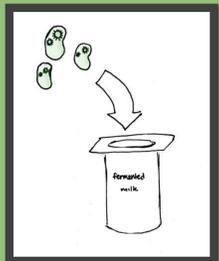
PROBLEM

- Iron deficiency affects 2 billion people, or over 30% of the world's population.
- Deficient populations may not lack in intake of iron, but rather lack in the intake of *bioavailable* iron.
- Phytic acid is a prevalent chelator of iron and other nutrients in food, rendering them unavailable for absorption.



IMPLEMENTATION

- Hydrolyze phytic acid with phytase, releasing chelated nutrients.
- Develop novel phytase delivery system utilizing fermented milk products native to the culture.



PHYTASE SYNTHESIS

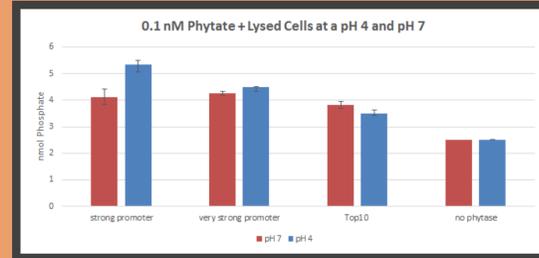
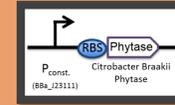
- Clone four different types of phytase from four different organisms: *Escherichia coli*, *Aspergillus niger*, *Bacillus subtilis*, and *Citrobacter braakii*.

PROTEIN RELEASE

- Engineer a probiotic that lyses and releases pre-synthesized phytase only when the probiotic is exposed to the acidic environment of the stomach (pH ~1.35-3.5)

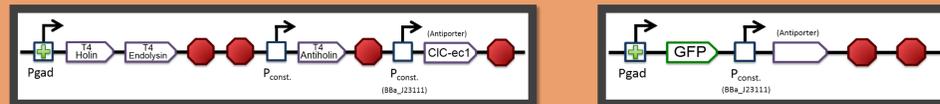
PHYTASE SYSTEM

- Citrobacter* phytase grown in *E. coli* liberated phosphate from the phytic acid solution.

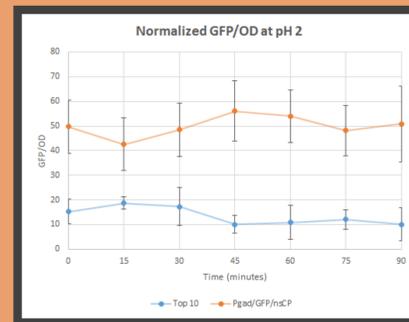
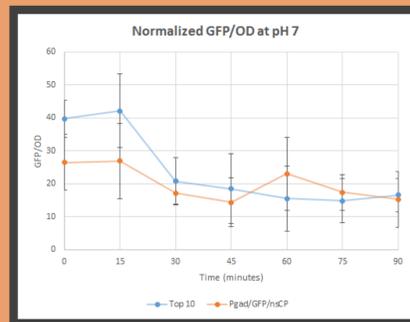


0.1 nM phytic acid was added to the sonicated cell lysate at both a pH of 7 and a pH of 4.5. At pH 4.5, lysed cells with phytase system demonstrate a higher phytase activity due to higher amount of free phosphate cut from the phytate.

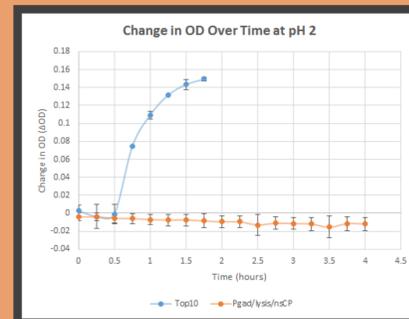
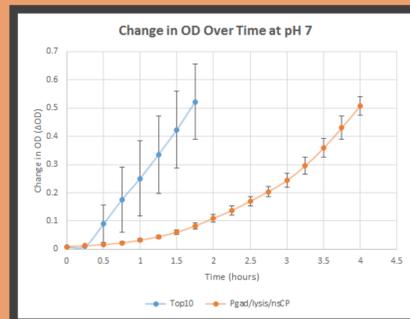
pH SYSTEM



- CIC antiporter imports chloride ions in exchange for protons.
- Pgard promoter is induced when chloride concentration rises.



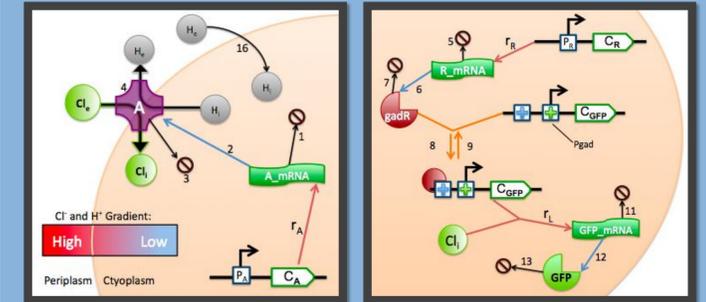
At pH 7, cells with the pH-inducible system have the same fluorescence as top10 cells (control). At pH 2, the cells have a higher fluorescence than the control.



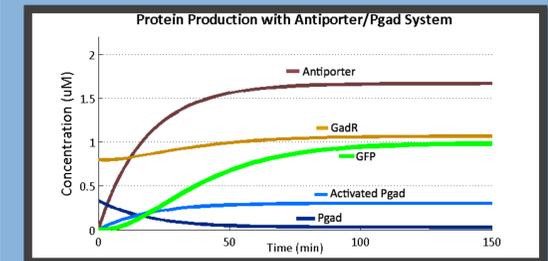
At pH 7, cells with the pH-inducible system grow considerably slower than control. At pH 2, the cell population noticeably decreases in comparison to the control.

MODELING

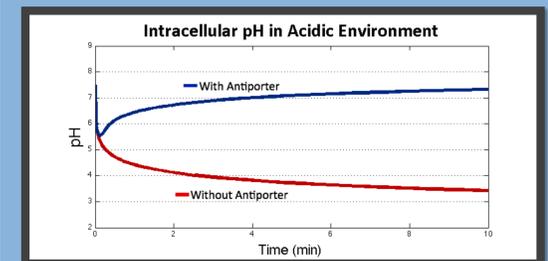
- Designed a model that simulates *Phytastic* cells entering the stomach to examine the plausibility of using the system for nutritional purposes.



System of ODEs was created using species from the antiporter (left) and Pgard/GFP (right) components.



GFP concentration rises as chloride levels increase and Pgard becomes activated. GFP reaches target concentration (1µM) within 2.5 hours.



Intracellular pH is affected by H⁺ influx and its transport out of the cytoplasm by the antiporter. The model shows cells quickly responding to dropping intracellular pH. Stability is restored within minutes.

CONCLUSION

- Phytase system produces phytase within the cell.
- Releasing the phytase demonstrates significant phytase activity.
- pH-inducible system demonstrates strong induction of both GFP and lysing of cells when introduced to a low-pH environment

ACKNOWLEDGEMENTS

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CITATIONS

Phytic acid chelation image: Syed S. Sohail and David A. Roland, Sr., Fabulous Phytase: Phytase Enzyme Proving Helpful to Poultry Producers and Environment. Highlights of Agricultural Research, 1999 46.
Kefir image: Fermented Milk. Discover the Incredible Health Benefits of Kefir. 11 October 2012. <http://products.mercola.com/body-ecology/kefir.htm>