



# Iron-enriched *Aspergillus oryzae* as an alternative to iron sulfate to limit iron accumulation, growth, and motility of the enteric pathogen *S. Typhimurium*

Manju B. Reddy,<sup>1</sup> Katelyn M. Miller,<sup>2</sup> David Quashie, Jr.,<sup>2\*</sup> Frank J. Velez,<sup>2</sup> Jamel Ali,<sup>2\*</sup> Prashant Singh,<sup>2</sup> Stephen R. Hennigar<sup>2</sup>  
<sup>1</sup>Department of Food Science and Human Nutrition, Iowa State University; <sup>2</sup>Department of Nutrition and Integrative Physiology, Florida State University

## Abstract

Excess unabsorbed iron in the gastrointestinal tract may select for enteric pathogens and increase the incidence and severity of infectious disease. *Aspergillus oryzae* (Ao) is a filamentous fungus that has the ability to accumulate and store large amounts of iron, and when used as a supplement or fortificant, has similar absorption to ferrous sulfate in humans. The objective of this study was to determine the effect of iron-enriched Ao (Ao iron) compared to ferrous sulfate (FeSO<sub>4</sub>) on iron accumulation, growth and motility of the Gram-negative enteric pathogen, *S. Typhimurium*. In the current study, *S. Typhimurium* was cultured in media containing no added iron or 1 μM elemental iron as either Ao iron or FeSO<sub>4</sub>. Results showed that *S. Typhimurium* cultured with FeSO<sub>4</sub> accumulated more iron than those cultured with Ao iron. Genes regulated by the iron-activated transcriptional repressor, Fur, did not differ between control and Ao iron, but decreased in *S. Typhimurium* cultured with FeSO<sub>4</sub> compared to both groups. Growth of *S. Typhimurium* was greater when cultured with FeSO<sub>4</sub> compared to Ao iron and control. *S. Typhimurium* swam faster, had greater acceleration, and traveled further when cultured with FeSO<sub>4</sub> compared to Ao iron and control; swim speed, acceleration, and distance traveled did not differ between Ao iron and control. These results provide evidence that Ao iron reduces the virulence of a common enteric pathogens and suggest that iron-enriched Ao may be a suitable iron supplement to improve iron delivery in areas with a high infection burden.

## Introduction

- The WHO recommends daily iron supplementation for infants and children and for women of reproductive age. Safety concerns have been raised with these recommendations in areas that have a high infection burden [1].
- Based on the low cost and high bioavailability and efficacy, iron sulfate (FeSO<sub>4</sub>) is typically the first choice for supplementation and fortification.
- The recommended dose of iron is set high to deliver adequate absorbed iron due to low rates of dietary iron absorption (<10%) [2]. Thus, most dietary iron is not absorbed and travels to the colon.
- Unabsorbed iron in the colon may select for enteric pathogens at the expense of beneficial commensal bacteria and increase infection risk [2].
- *Aspergillus oryzae* (Ao) is a filamentous fungus that accumulates and stores large amounts of iron. Recent research has focused on Ao as a vehicle for iron supplementation and fortification.
- Ao grown in FeSO<sub>4</sub> has been shown to have similar absorption to FeSO<sub>4</sub> in women of reproductive age with low iron stores [3].

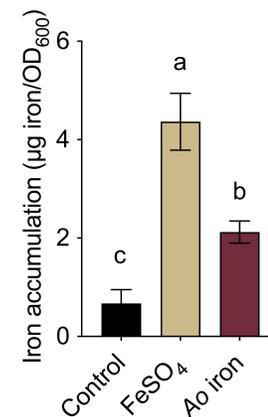
## Objective

The objective of this study was to determine the effect of iron-enriched Ao (Ao iron) compared to FeSO<sub>4</sub> on iron accumulation, growth and motility of the Gram-negative enteric pathogen, *S. Typhimurium*. *S. Typhimurium* is a motile facultative pathogen whose greatest burden is in regions where children are most effected by environmental enteric dysfunction [4].

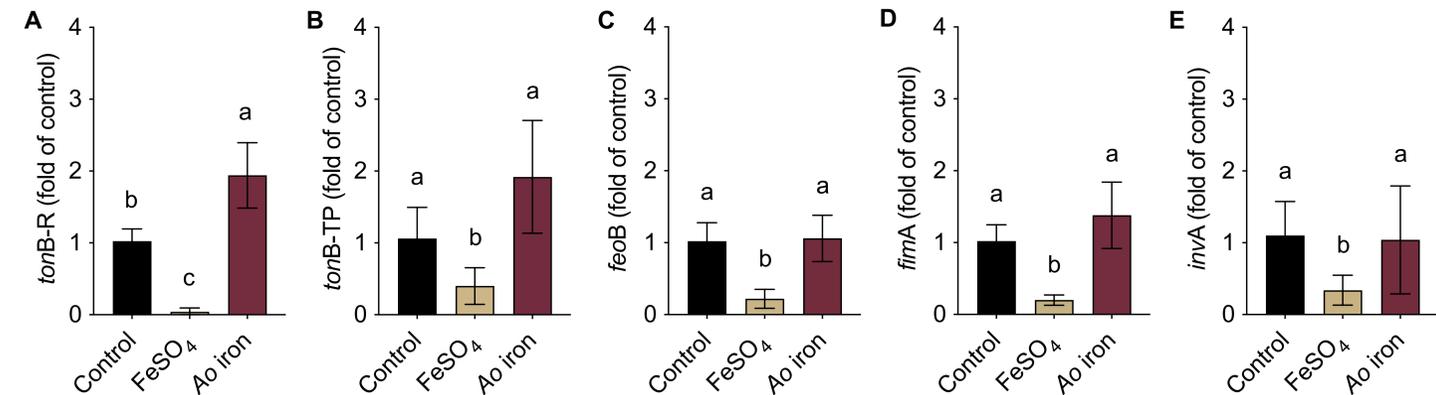
## Methods

- *S. enterica* subsp. *enterica* serovar Typhimurium ATCC® 14028™ were cultured in Iscove's Modified Dulbecco Medium (IMDM) containing:
  - No iron (Control)
  - 1 μM elemental iron as FeSO<sub>4</sub> (Sigma-Aldrich)
  - 1 μM elemental iron as FeSO<sub>4</sub>-enriched Ao (Cura Global Health Inc.)
- Unless noted otherwise, data were analyzed using a one-way ANOVA and different letters indicate a significant post-hoc comparison (P<0.05).
- Data are means ± SD. Experiments were repeated at least twice.

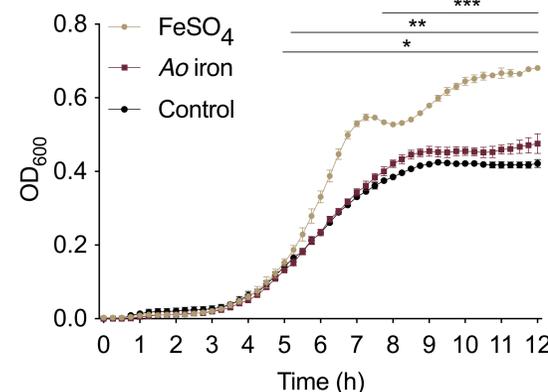
## Results



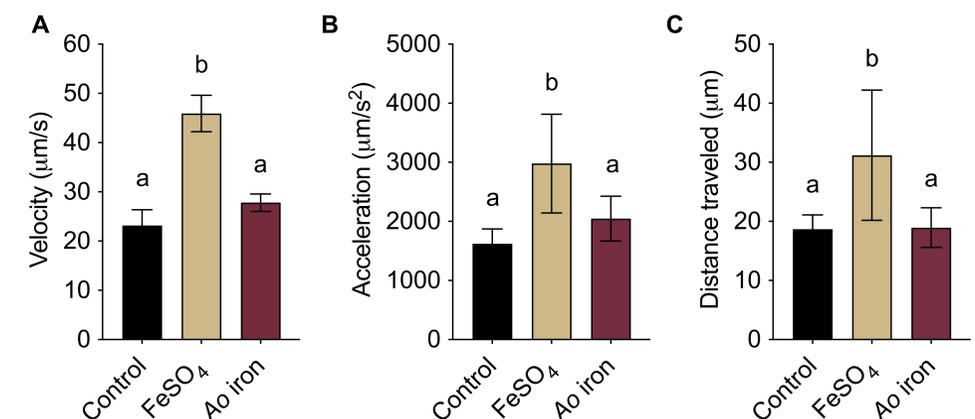
**Figure 1. Iron accumulation is reduced in *S. Typhimurium* cultured with Ao iron compared to FeSO<sub>4</sub>.** *S. Typhimurium* were cultured for 12 h and the iron concentration of digested bacteria was determined by flame AAS; n=3/treatment.



**Figure 2. Genes regulated by the iron-activated transcriptional repressor, Fur, are upregulated in *S. Typhimurium* cultured with Ao iron compared to FeSO<sub>4</sub>.** *S. Typhimurium* were cultured for 12 h and expression of A) *tonB-R*, B) *tonB-TP*, C) *feoB*, D) *fimA*, and E) *invA* were determined by RT-qPCR. Data are normalized to 16S and fold change was calculated using the  $\Delta\Delta Cq$  method; n=5/treatment.



**Figure 3. Ao iron restricts the growth of *S. Typhimurium* compared to FeSO<sub>4</sub>.** Growth of *S. Typhimurium* was determined by measuring OD<sub>600</sub> every 15 min for 12 h on a plate reader. Data were analyzed using a two-way ANOVA. Asterisks (\*) indicate a significant post-hoc comparison (P<0.05): \*P<0.05 FeSO<sub>4</sub> compared to Ao iron; \*\*P<0.05 FeSO<sub>4</sub> compared to control; \*\*\*P<0.05 Ao iron compared to control; n=3/treatment/timepoint.



**Figure 4. Ao iron restricts the motility of *S. Typhimurium* compared to FeSO<sub>4</sub>.** A) Velocity, B) acceleration, and C) distance traveled of *S. Typhimurium* cultured for 7 h. Phase contrast imaging was used to visualize bacterial motion, and recorded via high-speed camera. Videos were analyzed using 2D tracking algorithms using NIS-Elements AR Analysis software; n=5/treatment.

## Conclusions

- Ao iron restricts iron accumulation and virulence of the common Gram-negative enteric pathogen, *S. Typhimurium*.
- These findings suggest that Ao iron may be an effective alternative to FeSO<sub>4</sub> to address iron deficiency in areas with a high infection burden.
- The nature of Ao iron after digestion, mechanism of Ao iron absorption, and whether Ao iron is a cost-effective strategy is required to build the adoption of Ao iron as a supplement or fortificant in areas with a high infection burden.

\*Department of Chemical and Biomedical Engineering, FAMU-FSU College of Engineering.

## References

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