Excess unabsorbed iron in the gastrointestinal tract may select for enteric pathogens and increase the incidence and severity of infectious diseases. 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 sulfates in humans. The objective of this study was to determine the effect of iron-enriched Ao (Ao iron) compared to ferrous sulfate (FeSO₄) 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 FeSO₄. Results showed that S. Typhimurium cultured with FeSO₄ 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 FeSO₄ compared to both groups. Growth of S. Typhimurium was greater when cultured with FeSO₄ compared to Ao iron and control. S. Typhimurium swam faster, had greater acceleration, and traveled further when cultured with FeSO₄ 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 pathogen 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 (FeSO₄) 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 FeSO₄ has been shown to have similar absorption to FeSO₄ 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 FeSO₄ 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 affected by environmental enteric dysfunction [4].

### Methods

- **S. enterica subsp. enterica serovar Typhimurium ATCC® 14028** were cultured in Iscove's Modified Dulbecco Medium (IMDM) containing:  
- 1 µM elemental iron as FeSO₄ (Sigma-Aldrich)
- 1 µM elemental iron as FeSO₄-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 in S. Typhimurium cultured with Ao iron compared to FeSO₄.

- 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 FeSO₄. S. Typhimurium were cultured for 12 h and expression of A) tonB-R, B) tonB-TP, C) fer, D) fimF, and E) invA were determined by RT-qPCR. Data are normalized to 16S and fold change was calculated using the ΔΔCq method; n=5/treatment.

#### Figure 3. Ao iron restricts the growth of S. Typhimurium compared to FeSO₄. Growth of S. Typhimurium was determined by measuring OD₅₆₀ 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 FeSO₄ compared to Ao iron; ***P<0.05 FeSO₄ 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 FeSO₄. 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 NIH-Elements AR Analysis software; n=5/treatment.

### Conclusion

- 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 FeSO₄ 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.

### References


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