

EXPLORING IRON SCAVENGING AS AN UNDER-EXPLORED MODE FOR PATHOGEN ELIMINATION BY *BACILLUS*-BASED PROBIOTICS

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INTRODUCTION

- ✓ Iron is an essential nutrient for the survival of aerobic organisms, but its bioavailability is limited in the gut.
- ✓ Microbes produce siderophores to scavenge iron, with some pathogens being more iron-dependent than beneficial microbes. Iron promotes replication and virulence in pathogens¹.
- ✓ *Bacillus*-based probiotics inhibit pathogens by secreting antimicrobial secondary metabolites².
- ✓ The supplementation of a novel *Bacillus licheniformis* G3 (ATCC PTA-127113) in broiler birds reduced the prevalence of *Salmonella* in the ceca and crop under challenged conditions in a prior study.
- ✓ The current study investigated the iron scavenging effect of the siderophores produced by the *Bacillus licheniformis* G3 (ATCC PTA-127113) and its impact on *Salmonella*.

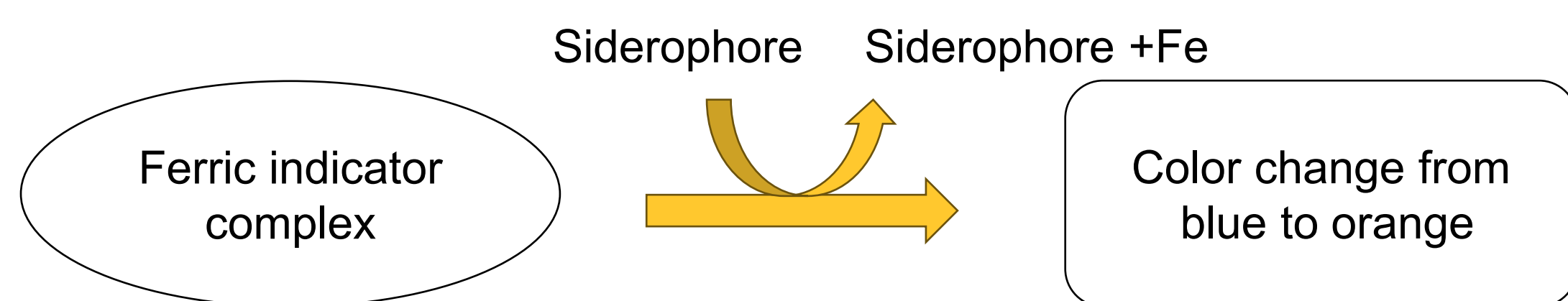
MATERIALS AND METHODS

➤ Identification of Gene Clusters.

- Whole genome of the *Bacillus licheniformis* G3 (ATCC PTA-127113) was sequenced using Illumina sequencing and the secondary metabolite gene cluster identification was done using Bacterial antiSMASH³.

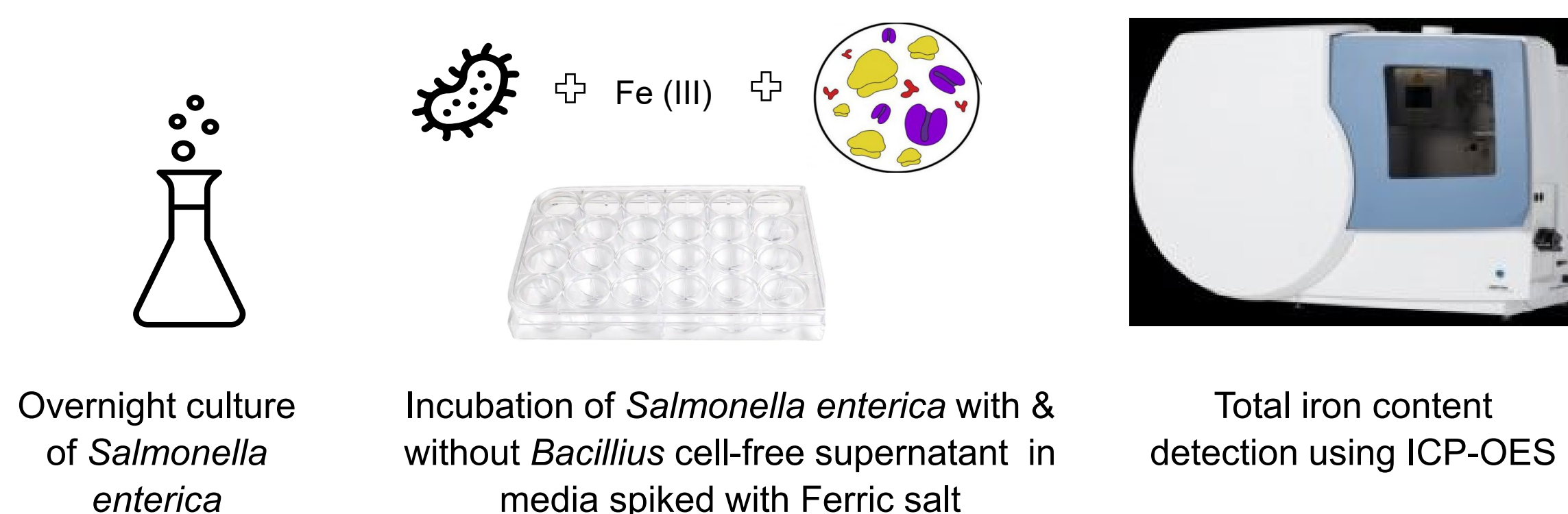
➤ Siderophore Detection Assay.

- Chrome azurol S (CAS) colorimetric assay⁴.



- The cell-free supernatant (CFS) of a freshly grown culture of *Bacillus licheniformis* G3 (ATCC PTA-127113) was incubated with an equal volume of CAS reagent for 20 mins, and optical density was measured at 630nm.

➤ Iron Binding Estimation.



RESULTS

- **Gene cluster identification.** Gene clusters of siderophores, high-affinity iron chelating metabolites, were identified by the antiSMASH tool. Hydroxamate and catecholate classes of siderophores were recognized in the gene clusters.

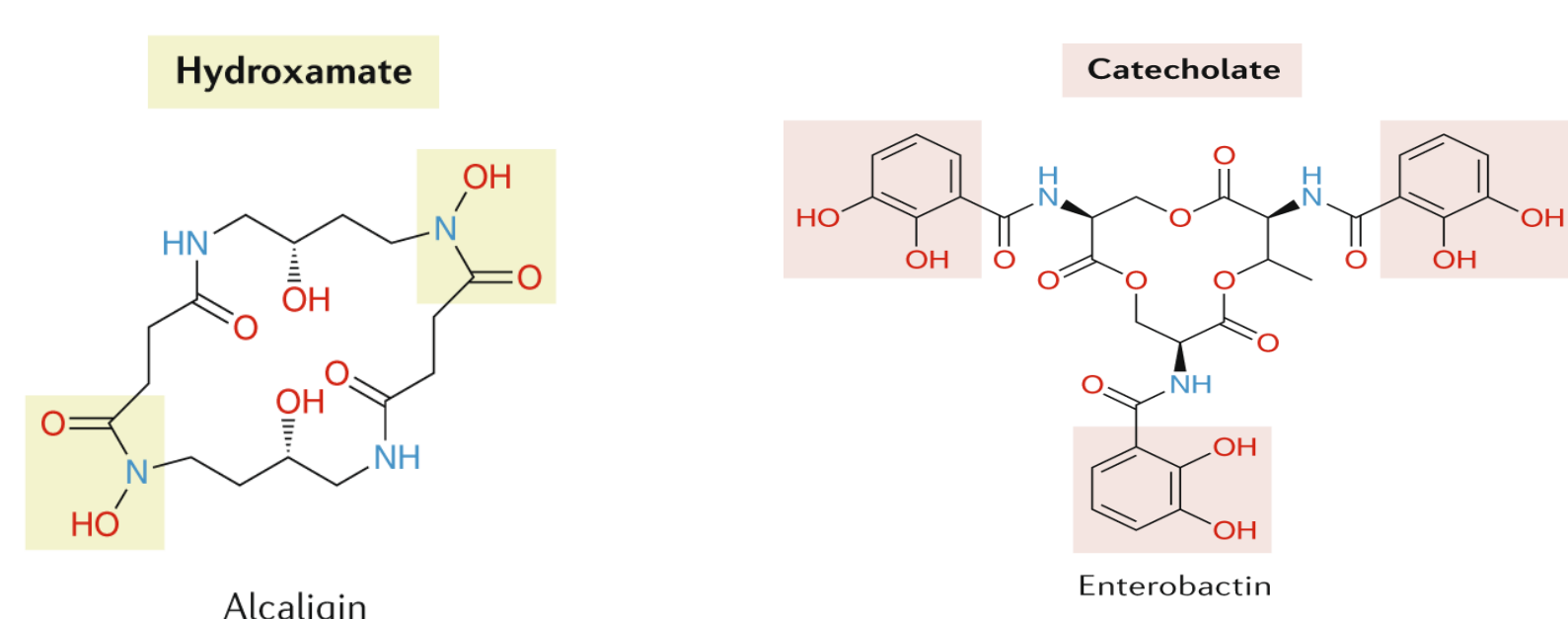


Fig. 1. Examples of hydroxamate and catecholate siderophores with highlighted active sites⁵

➤ Siderophore detection by CAS assay.

- The presence of siderophores in the *Bacillus licheniformis* G3 (ATCC PTA-127113) was detected through a color change in the ferric indicator complex.
- Quantitatively, 38% iron chelation was observed in the CFS of the *Bacillus* strain.
- This was higher than the iron binding percentage of standard strains of *E. coli* (6%) and *Salmonella enterica* (0%)



Fig. 2. Siderophore detection in *Bacillus licheniformis* G3 (ATCC PTA-127113) by iron chelation using CAS assay

➤ Iron binding by *Bacillus licheniformis* G3 (ATCC PTA-127113) incubated with *Salmonella enterica*.

- The total iron content in the culture supernatant of *Salmonella enterica* with *Bacillus licheniformis* G3 (ATCC PTA-127113) CFS was lower compared to the control without CFS, indicating iron scavenging by the siderophores.
- The iron content decreased linearly as the ferric salt concentration in the media increased, indicating competitive iron binding.
- The growth of *S. enterica* was not affected by high iron concentrations, confirming that the iron levels used are not toxic to *S. enterica*.

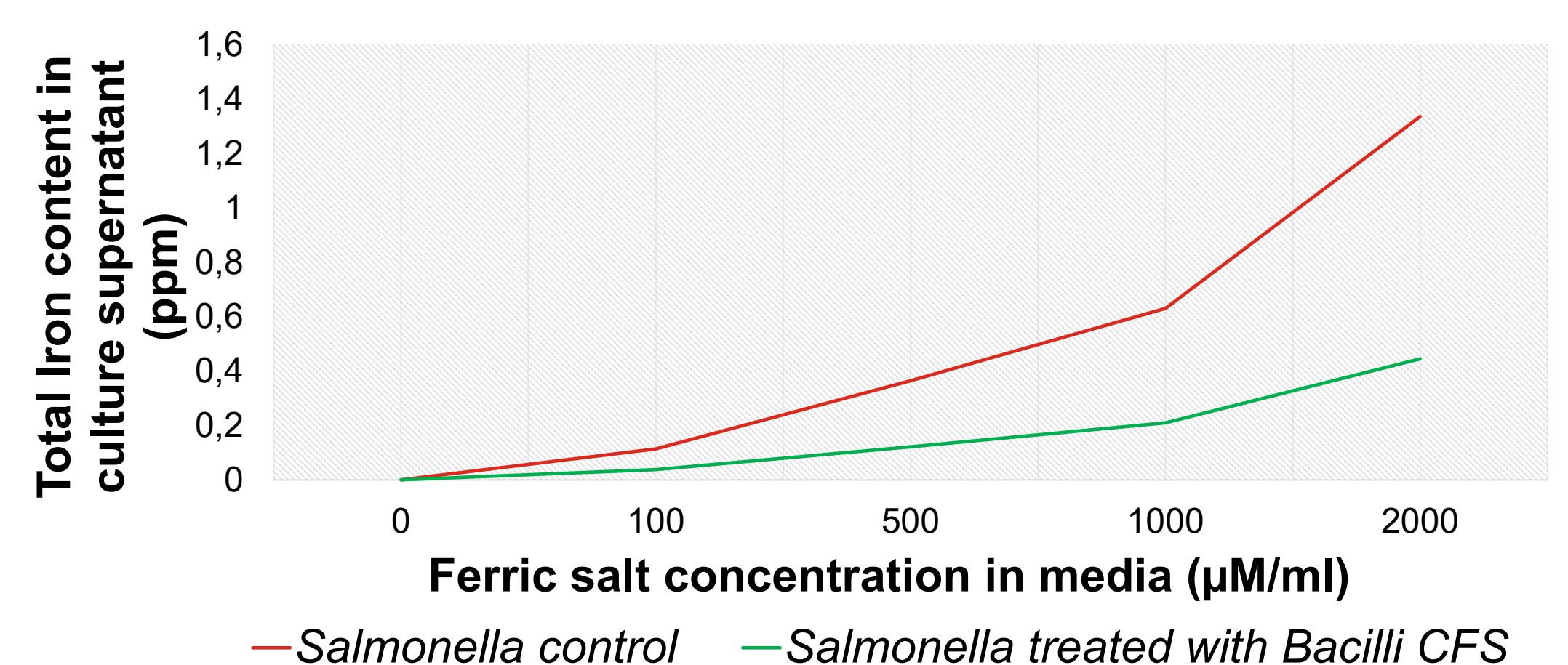


Fig. 3. Estimation of iron binding by *Bacillus licheniformis* G3 (ATCC PTA-127113) incubated with *Salmonella enterica* using ICP-OES

DISCUSSION AND CONCLUSION

- The novel *Bacillus licheniformis* G3 (ATCC PTA-127113) strain exhibited gene clusters of high-affinity iron-scavenging siderophores which was confirmed by quantitative CAS assay.
- The iron scavenging ability of the siderophores was confirmed by the reduction in the iron content in the culture supernatant of *Salmonella enterica* treated with the CFS of the *Bacillus* strain.
- The reduced availability of iron for the virulence of *Salmonella* by siderophores might be well substantiated in in-vivo conditions or through cell culture studies.
- Further exploration of *Bacillus*-based siderophores in regulating host iron homeostasis against other enteric pathogens may unveil their pivotal role in conferring an advantage over pathogens.

References:

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