Exploring iron scavenging as an under-explored mode for pathogen elimination by *Bacillus*-based probiotics

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Iron, an indispensable nutrient, vital for the survival of nearly all aerobic organisms, acts as a catalyst in cellular redox reactions, aiding DNA synthesis, energy production, and various metabolic processes. Its bioavailability, however, is often limited particularly in the gut environment, creating a competitive struggle among microbial species. To address this scarcity, microbes secrete small iron-scavenging compounds such as siderophores, that have a high affinity to iron and support the sequestration and solubilization of ferric iron from the host environment. Some of the common gut enteric pathogens such as *Salmonella*, *E. coli, Shigella,* and *Clostridium* are more iron-dependent than beneficial gut microbes such as *Lactobacilli*. Iron promotes replication and virulence in these pathogens. The antimicrobial effects of *Bacilli* strains against these pathogens have been well reported, majorly by the production of secondary metabolites with direct inhibitory effects. The current study aimed to explore the less-studied effect of siderophores from a novel *Bacillus licheniformis* against *Salmonella*. The *B. licheniformis* reduced the prevalence of *Salmonella* in salmonellosis-infected broiler birds. The genomic mining of the *Bacilli* revealed the presence of several siderophore gene clusters. The in vitro iron binding capacity of the *Bacilli* was confirmed by chrome azurol S (CAS) assay and compared with that of the poor binding capacity of *E. coli* and *Salmonella* enterica. Further, the preferential iron binding capacity of the cell-free supernatant (CFS) of the *Bacilli* strain was tested by incubating it with Salmonella enterica in a medium spiked with ferric salt. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) analysis of the spent media revealed decreased iron concentration in the group supplemented with CFS, indicating reduced iron availability for *Salmonella* growth, possibly due to iron chelation by the CFS. These findings suggest the positive role of siderophores in combating *Salmonella's* iron-dependent pathogenicity. Further exploration of *Bacilli*-based siderophores in regulating host iron homeostasis against other enteric pathogens may unveil their pivotal role in conferring an advantage over pathogens.

KEYWORDS:
Iron scavenging, *Bacillus licheniformis*, Siderophores, *Salmonella*.