

Title:

Inhibition of *Legionella* spp. through siderophores-mediated iron scavenging

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Abstract (300 words maximum):

Several species within the genus *Legionella* are human opportunistic pathogens ubiquitous in freshwater and often found in engineered aquatic ecosystems. Conventional control of *Legionella* include water treatments with high temperature and disinfectants (e.g. chlorine), which are sometimes not effective. The elevated iron requirements suggested in literature for *Legionella* offer a possibility to reduce their presence by inducing iron starvation, specifically using siderophores. Siderophores are secreted secondary metabolites that scavenge ferric iron from the environment, and have previously been shown to be inhibitory towards other human pathogens. Here, the effect of variable iron concentrations on the growth of five *Legionella* species was examined, and the inhibitory potential of different siderophores produced by *Pseudomonas* spp. explored. Specifically, five non-pathogenic environmental *Pseudomonas* isolates were selected based on their production of highly potent pyoverdines whose action was tested in growth assays against *L. pneumophila*. The results revealed that iron limitation mainly affected the lag-phase of *Legionella* spp., with an evident shift between iron concentrations of 5 and 17 μM , although interspecies differences were observed, which suggest variable iron requirements or acquisition mechanisms among *Legionella* spp. (lag phase ranging 30-80 hours among species in the absence of added iron). Moreover, the pyoverdines of *Pseudomonas* isolates were all demonstrated to be active against *L. pneumophila*, successfully affecting its growth by inducing iron starvation, with effects that include differences of up to 55 hours in the lag-phase or complete growth inhibition. These results suggest that iron concentrations may affect both the abundance and diversity of *Legionella* spp. in the environment, and the potential of iron starvation as an alternative biological strategy against *Legionella* in aquatic ecosystems.