Bacteria fight club: Identifying probiotics for goldfish

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Exposure to pollutants, like nitrite, negatively impacts fish both directly and indirectly. Nitrite can cause brown blood disease in fish when they are exposed to elevated nitrite concentrations. This occurs when hemoglobin is converted into methemoglobin which cannot transport oxygen. Although the direct effects of nitrite are well studied, it is unknown how nitrite impacts the microbiome. The microbiome is important because it supports the immune system by taking resources from pathogens and competing directly with them using antimicrobial molecules. When the microbiome is disrupted, it can lead to increased susceptibility to disease and disturbed physiological functions, which can lead to death. Probiotics, a microorganism administered for its beneficial attributes, are often used to stabilize a disrupted microbiome. The added bacteria are specifically selected to correct an imbalance by recolonizing the microbiome, releasing useful metabolites, and competing with pathogens. The impact of elevated nitrite concentrations on fish is important because they are a major food source. However, when they are farmed in aquaculture, they are exposed to elevated levels of nitrite. This study aimed to identify probiotic candidates that could be used to stabilize the fish microbiomes after they were exposed to elevated concentrations of nitrite. To accomplish this, bacteria were isolated from the tissues of healthy goldfish by swabbing with sterile swabs, growing on ½ BHI or R2A media, and identified using Sanger sequencing. A competition and agar plug assay were then used to determine the antimicrobial properties of the candidates against *Yersinia ruckeri*, *Edwardsiella ictaluri*, *Vibrio harveyi*, and *Aeromonas hydrophilia* which are common fish pathogens in aquaculture. The pathogens were plated with the probiotic candidates to see which candidate inhibited the growth of the pathogens. Of the 22 candidates tested, 15 were able to inhibit the growth of at least one pathogen on an agar plug or competition assay. The candidates were then exposed to the same tests with nitrite in the media to identify those that could inhibit pathogen growth when grown with nitrite. The two best candidates were a *Pseudomonas* and *Pseudoxanothomonas* species. These bacteria were isolated from healthy goldfish and should be safe while also inhibiting pathogen growth. Probiotics have the potential to mitigate the negative effects of nitrite exposure in fish, and bacteria isolated from healthy fish should not pose a risk. These probiotics could be used in aquaculture to reduce the cost and increase fish production. While probiotics have been used to stabilize the microbiome, there has been no research into how probiotics could be used to stabilize the microbiome from nitrite exposure. Additionally, this probiotic would be used while the fish are still being exposed to elevated nitrite levels and would have to both thrive and prevent pathogen growth at these higher levels. This research, therefore, is investigating the potential benefits of unexplored probiotics that have the potential to drastically help aquaculture production.