

HUERTAS LAB
FISH PATHOLOGY AND PREVENTION

Bacteria fight club: Identifying probiotics for goldfish

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Introduction

- Aquaculture supports both the economy and food availability in poor regions (1).
- Elevated nitrite concentrations, common in aquaculture, disrupt fish microbiome, which potentially can induce illnesses.
- Probiotics can be used to stabilize the microbiome.

We identified probiotic candidates with the goal of identifying a bacteria that can prevent pathogen growth while being safe for the fish

Methods

- Bacteria isolated from the tissue microbiomes of healthy goldfish identified using Sanger sequencing.
- Probiotic candidates were tested for antimicrobial ability using an agar plug and competition assay.
- Successful candidates were retested using the same assays with nitrite infused media.
- Top candidates were selected based on the antimicrobial activity and genus characteristics.

Results

| Code | Strain Code | Source | Genus | EI | VH | YR | AH |
|------|-------------|--------|-------------------|-----|-----|-----|----|
| S1 | S00367 | Skin | Aeromonas | Yes | No | No | No |
| G1 | S00359 | Gill | Cellulomonas | Yes | Yes | No | No |
| G2 | S00368 | Gill | Aeromonas | Yes | No | No | No |
| N1 | S00553 | Nose | Chitinilyticum | Yes | Yes | No | No |
| N2 | S00551 | Nose | Aeromonas | Yes | No | Yes | No |
| N3 | S00557 | Nose | Pseudoxanthomonas | Yes | Yes | Yes | No |
| N4 | S00541 | Nose | Contaminated | Yes | No | No | No |
| N5 | S00544 | Nose | Pseudomonas | Yes | No | No | No |
| N6 | S00542 | Nose | Pseudomonas | Yes | No | Yes | No |

Table 1. Summary of probiotic candidates derived from the skin, gills, and nose of healthy goldfish. A direct competition assay and an agar plug assay were conducted to determine if the candidates had any antimicrobial activity against for pathogens, *E. ictaluri* (EI), *V. harveyi* (VH), *Y. ruckeri* (YR), and *A. hydrophilia* (AH).

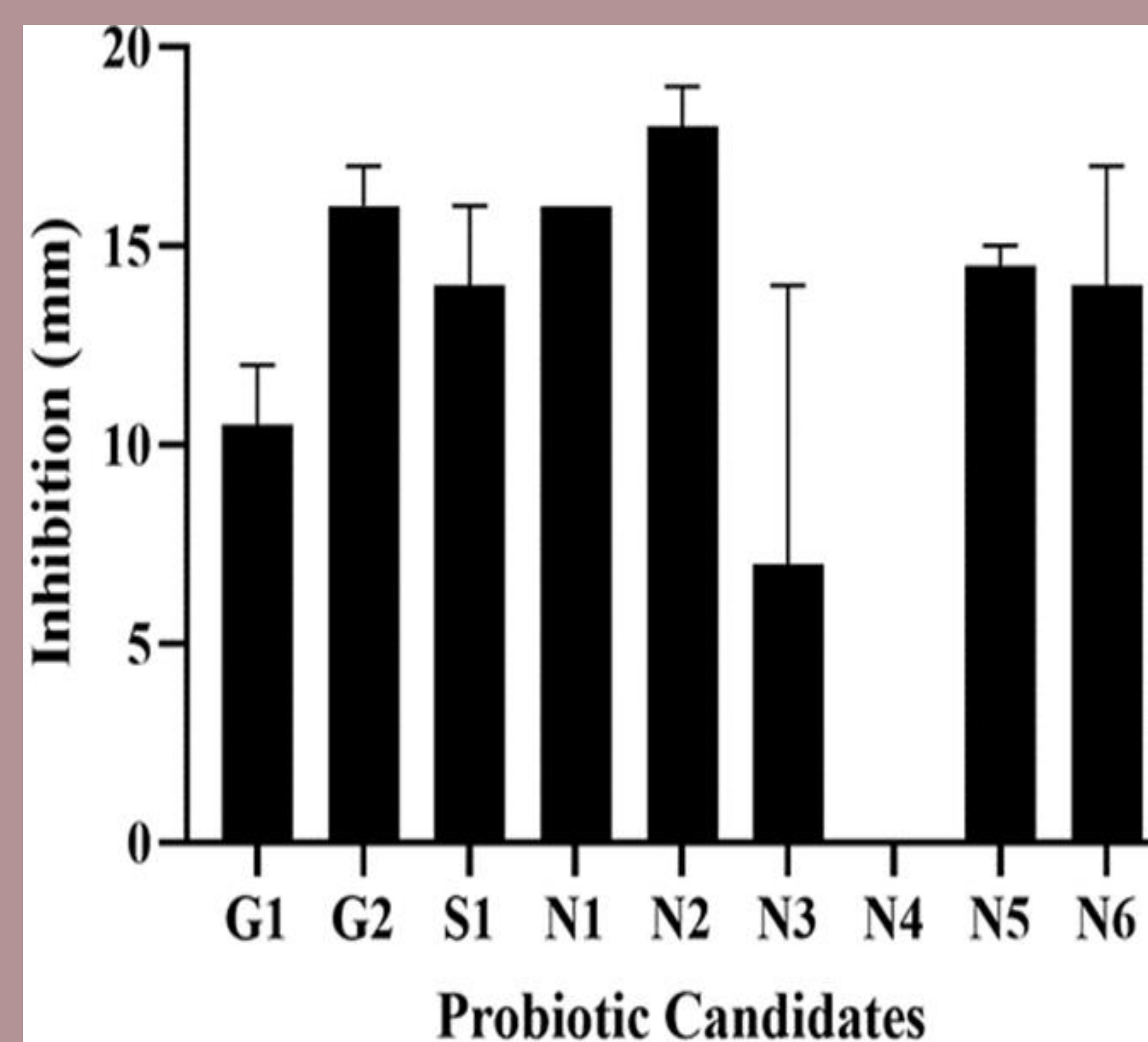


Figure 1. Inhibition of plated on ½ BHI by probiotic candidates using an agar plug assay (mean ± SEM). The zones of inhibition shown are against *E. ictaluri* when with no added nitrite.

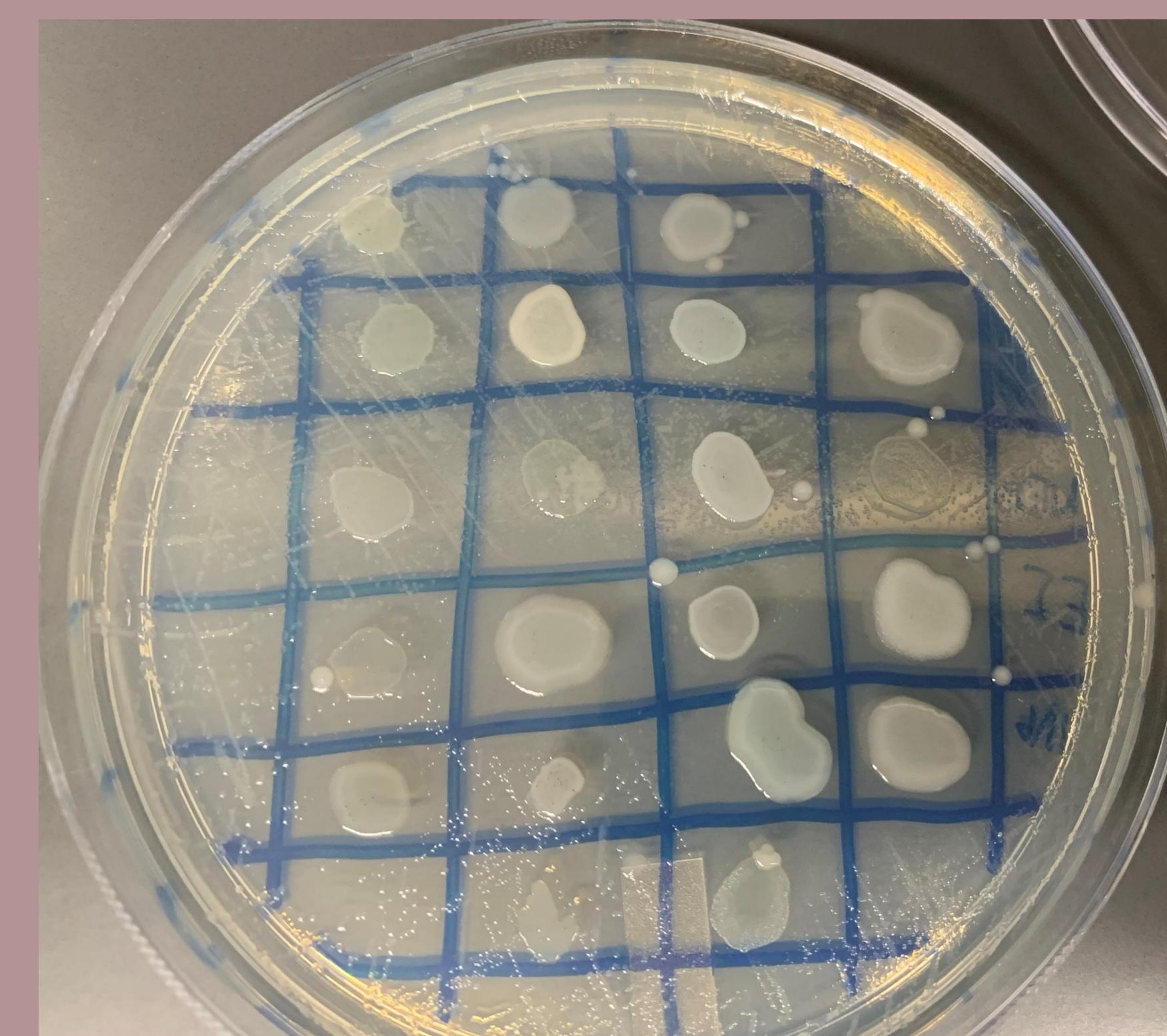


Figure 2. Example of direct competition assay plate featuring *E. ictaluri* as the pathogen.

Discussion

- Most bacteria isolated from the goldfish microbiomes possessed some antimicrobial ability.
- Nitrite inhibited the growth of pathogens *in vitro*, despite being more common in farm fish exposed to nitrite (2).
- Candidates N3 and N5 were determined to be the best potential probiotics.
- Species isolated from the microbiome should pose little risk to the fish while stabilizing the microbiome (3).
- Future works should test these candidates *in vivo* to confirm their safety and efficacy in fish exposed to nitrite.

References

1. Tacon, A. (2020) Trends in global aquaculture and aquafeed production: 2000-2017. Reviews in Fisheries Science & Aquaculture 28: 43-56.
2. Lai, W.W.P., Lin, Y.C., Wang, Y.H., Guo, Y.L., and Lin, A.Y.C. (2018) Occurrence of emerging contaminants in aquaculture waters: Cross-contamination between aquaculture systems and surrounding waters. Water Air and Soil Pollution 229: 1-12.
3. Lara-Flores, M. (2011) The use of probiotic in aquaculture: An overview. International Research Journal of Microbiology 2: 471-478.