

This research investigates the use of probiotics in farm fishing to offset the the industry's reliance on antibiotics and other antimicrobials. Probiotics may serve as a preventative measure to disease as opposed to a treatment for disease symptoms.

# Identification and Isolation of Novel Probiotic Bacteria for use in Marine Aquaculture

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## Who cares and why?

With the continuing collapse of US commercial fisheries and the escalating disparity between supply and demand for fishery products, aquaculture will increasingly be relied upon to meet the national and global demand for seafood. As disease is a major problem for the fish farming industry, many strategies have been taken to overcome this obstacle, including the addition of antibiotics chemotherapeutics. While these approaches have met with some success, they have their problems. The presence of high levels of antibiotics in farmed fish and the concern about antibiotic-resistant organisms have led to the understanding that the emphasis in disease management should be on prevention, rather than a cure. Applying preventive measures may lead to less reliance on the use of chemicals-disinfectants. pesticides and

antimicrobials - that treat the symptoms of the problem rather than affecting a cure.

One strategy for controlling disease has been to utilize probiotics, which are live microbial supplements that beneficially affect the host by modifying the host-associated microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response to disease, or by improving water quality of its ambient environment. The probiotic acts by either competing with other bacteria for essential resources or nutrients, antagonism, or by producing their own broad-spectrum antibiotics. The application appears to be useful in a wide range of life-history stages, from larvae to adults. In the aquaculture industry, the application of probiotics is not systematically used and little is known about the specific mechanisms used by individual probiotic bacteria for protection.

## What has the project done so far?

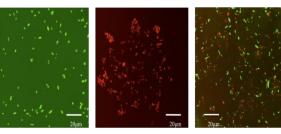
To optimize their effectiveness, probiotics should be selected from (adapted to) the environment in which they will be eventually used. *Fundulus heteroclitus* lives in habitats with a wide range of salinities, and can be cultivated in a similarly wide range in aquaculture. Therefore, the potential probiotics that we derived from this species as part of our 2012 NRAC Mini-grant have the potential to be applied to commercially important species from a range of salinities.

By utilizing *F. heteroclitus* as a source of potential probiotics, the candidate probiotics will similarly be applicable to a range of environmental conditions. Our ongoing work with oyster probiotics in conjunction with the NOAA Milford Aquaculture Lab, and funding from our 2012 NRAC Mini-grant have laid a solid foundation for the identification and application of novel species of probiotics for use in the Northeast (Fig. 1). Collectively, this work will expand our knowledge base in respect to probiotic bacteria, and thereby allow us to improve

aquaculture production through cost-effective management, reduce input costs and help improve

shellfish and finfish health maintenance and disease control.

Through our efforts to date, we identified seven non-Vibrio bacteria from the intestines of Fundulus heteroclitus that had the ability to inhibit growth of not only the fish pathogens Vibrio harveyi (DNO1) and Vibrio damsela but also may inhibit growth of Vibrio sp. B183 (a shellfish pathogen) as determined by filter disk assays. Of these, we tested four



**Fig. 1.** Epifluorescence images of *Vibrio* sp strains carrying genes for fluorescent proteins. Left, probiotic OY15g (with *gfp*); middle, pathogen B183r (with *rfp*); right, OY15g and B183r. (photo by Harold Schreier)

probiotic bacteria (OY15, Iso5, Iso11 and Iso12) selected by their ability to inhibit pathogen growth and a glycerol-only control (the probiotic storage medium) in a short-term growth trial with model species, heteroclitus, to ascertain the potential effects of the novel probiotic bacterial strains on the larvae.

## **Impact Statement**

The application of probiotics appears to be useful in a wide range of life-history stages, from larvae to adults, though in aquaculture, the application of probiotics is not systematically used and little is known about the specific mechanisms used by individual probiotic bacteria for protection..

Our research has identified seven non-Vibrio bacteria from the intestines of *Fundulus heteroclitus* that had the ability to inhibit growth of not only the fish pathogens *Vibrio harveyi* (DNO1) and *Vibrio damsela* but also may inhibit growth of *Vibrio* sp. B183 (a shellfish pathogen. Four of these bacterial isolates were subsequently tested on larval F. heteroclitis and some appear to also have growth enhancing effects.

#### What research is needed?

Future research will continue to evaluate these potential probiotic strains on the survival and growth of larvae of other commercially important aquaculture species.

### Want to know more?

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