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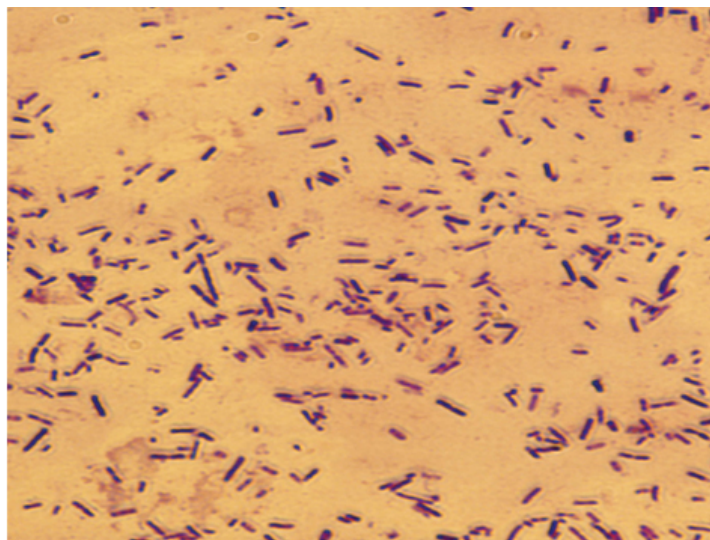
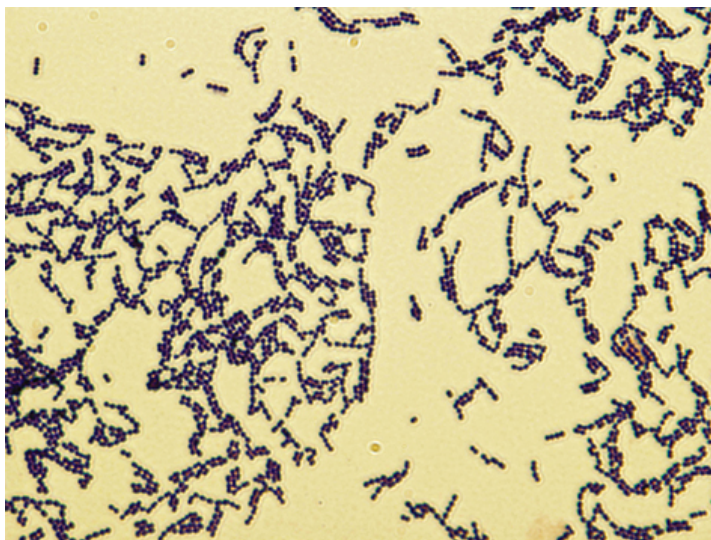
Developing live bacterial vaccines by selecting resistance to antibacterials



2 May 2014 Dr. Julia W. Pridgeon Dr. Phillip Klesius



With an increase in antibiotic comes greater need for alternative control methods



Major bacteria diseases affecting aquaculture include streptococcosis caused by Gram-positive sphere-shaped bacteria (left) such as *Streptococcus agalactiae* and *S. iniae*, and enteric septicemia caused by Gram-negative rod-shaped bacteria (right).

Aquaculture is the fastest-growing food-production industry in the world. However, diseases are plaguing this sector, causing economic losses estimated to be billions of dollars worldwide. Major bacteria diseases affecting aquaculture include streptococcosis caused by Gram-positive sphere-shaped bacteria such as *Streptococcus agalactiae* and *S. iniae*, enteric septicemia caused by Gram-negative rod-shaped bacteria such as *Edwardsiella tarda* and motile aeromonad septicemia caused by Gram-negative bacteria such as *Aeromonas hydrophila*.

To control fish bacteria diseases, feeding infected fish with approved, antibiotic-medicated feed is a general practice. However, such practice is expensive and usually ineffective, as sick fish do not like to eat. In addition, only three antibiotics are currently approved for use in aquaculture in the United States: oxytetracycline, sulfadimethoxine and florfenicol.

The widespread use of the limited number of antibiotics for treating bacterial diseases in aquaculture has led to the development of antibiotic resistance in many fish pathogens worldwide. Therefore, alternative control methods are urgently needed for the aquaculture industry.

Vaccines

The use of vaccines is an alternative control method to prevent bacteria diseases in aquaculture. The most extensively studied fish vaccines are bacterins consisting of formalin or heat-killed bacteria of pathogenic strains. In addition, recombinant protein vaccines and bacterial lysates have been demonstrated to elicit protection against challenge with virulent bacteria. Furthermore, live attenuated vaccines have been reported to confer significant protection against challenge with virulent bacteria.

To develop effective live bacterial vaccines, a rifampicin-resistant strategy has been successfully used to attenuate *E. ictaluri* and *Flavobacterium columnare*. However, it is not clear whether other chemicals could also be used to attenuate bacteria for the purpose of novel vaccine development.

Gossypol, a natural compound derived from the cotton plant, is known to have a mutagenic effect on DNA. Novobiocin and ciprofloxacin are antibiotics. Therefore, the objective of this study was to determine whether gossypol, proflavine hemisulfate, novobiocin or ciprofloxacin was effective in attenuating bacteria for the purpose of vaccine development.

Induction of resistance in bacteria

All bacteria used in the study developed 250- to 800-fold resistance to gossypol rapidly, with an initial selection concentration of 2 µg/mL. However, only 33 percent (6 of 18) and 50 percent (10 of 20) of the Gram-negative and Gram-positive bacteria developed resistance to proflavine hemisulfate, respectively.

The six Gram-negative bacteria developed 16- to 1,000-fold resistance to proflavine hemisulfate, whereas the 10 Gram-positive bacteria developed 20,000- to 80,000-fold resistance to proflavine hemisulfate. When novobiocin was used to select for resistance, Gram-negative bacteria as well as Gram-positive *S. agalactiae* isolates were able to develop resistance.

Gram-negative bacteria developed 64- to 2,000-fold resistance to novobiocin. Gram-positive *S. agalactiae* isolates developed 256- to 16,000,000-fold resistance to novobiocin. However, *S. iniae* was very sensitive to novobiocin. Only two *S. iniae* isolates developed 256,000- and 2,000,000-fold resistance to novobiocin, respectively. Similarly, when ciprofloxacin was used to select for resistance, all Gram-negative bacteria, as well as a majority of the *S. agalactiae* isolates, developed resistance, whereas only one *S. iniae* isolate was able to develop 128-fold resistance to ciprofloxacin.

Bacteria virulence to tilapia, catfish

All gossypol-resistant bacteria were either more virulent to tilapia or slightly less virulent than their respective parents. Of 20 proflavine hemisulfate-resistant bacterial isolates, 18 were found avirulent to tilapia by intraperitoneal injection. Of 18 novobiocin-resistant bacteria isolates, four were avirulent to channel catfish.

Of 13 Gram-positive bacteria that were resistant to novobiocin, three were avirulent to tilapia. Of 12 ciprofloxacin-resistant *S. iniae* or *S. agalactiae*, five were found to be avirulent. Of 11 ciprofloxacin-resistant bacterial isolates, three were avirulent to channel catfish.

Vaccinated tilapia, catfish challenged with isolates

All proflavine hemisulfate-resistant isolates failed to provide significant protection to Nile tilapia against challenges with virulent bacteria isolates, with relative survival less than 33 percent. Of nine novobiocin-resistant *S. iniae* or *S. agalactiae* isolates tested, two were found to offer 100 percent protection. Of seven ciprofloxacin-resistant *S. iniae* or *S. agalactiae* isolates tested, only two provided 88 and 63 percent protection.

Two novobiocin-resistant isolates were able to provide 100 percent protection to channel catfish against challenges with virulent bacteria isolate. Of five ciprofloxacin-resistant *A. hydrophila* or *E. tarda* isolates tested, three were found to offer 100 percent protection to channel catfish.

Larger-scale study

When vaccinated catfish were challenged by virulent *E. tarda* 30305, *E. tarda* 30305-novobiocin offered 100 percent protection to channel catfish. Similarly, when vaccinated Nile tilapia were challenged by virulent *E. tarda* 30305, relative percent of survival of vaccinated fish at 14- and 28-dpv was 100 percent and 92 percent, respectively. Cumulative mortalities of *E. tarda* 30305-novobiocin vaccinated fish at different time points were significantly lower than that of tryptic soy broth sham-vaccinated fish.

For backpassage safety, of all fish exposed to *E. tarda* 30305-novo vaccine through intraperitoneal injection, no mortality or signs of disease or adverse behavior was observed. No fish died in the backpassage safety studies after exposure to *E. tarda* 30305-novo.

Perspectives

Four chemicals were used in this study to modify 38 bacterial isolates through a chemical-resistance strategy. All bacteria used in this study were able to develop high resistance to gossypol. However, none of the gossypol-resistant isolate was attenuated. Although the majority of the proflavine hemisulfate-resistant isolates were attenuated, all of them failed to provide significant protection to fish.

Eight novobiocin- or ciprofloxacin- resistant Gram-positive bacteria were found to be attenuated. However, none of them offered protection higher than 70 percent. Of seven attenuated novobiocin- or ciprofloxacin-resistant Gram-negative isolates, only one (novobiocin-resistant *E. tarda* 30305) was found to be relatively safe and highly efficacious.

When *E. tarda* 30305-novo-vaccinated Nile tilapia were challenged by virulent *E. tarda* 30305, relative percent of survival of vaccinated fish at 14 and 28 days post vaccination was 100 percent and 92 percent, respectively. Similarly, *E. tarda* 30305-novo offered 100 percent protection to channel catfish against challenges with virulent parent isolate *E. tarda* 30305.

The results suggested that the development of live attenuated bacterial vaccines that are safe and efficacious is feasible, although it is challenging.

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