



Ten Years Of **Research By** Marineland Aquarium Products has resulted in the discovery of a new bacterium that shortens the time it takes to prepare a new aquarium for fish. For retailers. this could mean increased success for hobbyists, which could lead to more fish sales.

Complete story inside.

Discovery Could Be Boon to Fishkeeping

By **Kathy Johnston** Special to Pet Product News

A patented bacterium that users say dramatically shortens the break-in period for new aquariums will be released to retailers at the American Pet Products Manufacturers Association show in Chicago this month.

Marineland Aquarium Products has isolated and cultured the beneficial bacteria that biologically detoxify the ammonia and nitrite responsible for new-tank syndrome, according to the Moorpark, Calif. company's chief science officer, Timothy Hovanec, Ph.D.

The newly discovered bacteria will have major implications for the aquarium hobby, he said.

"It's always been a problem for retailers to tell customers to wait for weeks before adding a normal population of fish to their new aquarium," Hovanec said. "By adding this bacteria, they can put their fish in the next day, once the temperature stabilizes."

"If used properly, it will go a long way toward eliminating new-tank syndrome," said Dr. Paul Loiselle, curator of freshwater fishes at the New York Aquarium. The longtime hobbyist beta tested the bacteria in a new exhibition tank and in his home aquarium.

The combination of patented and patent-pending bacteria will be marketed by Marineland as BIO-Spira, named for the spiral shape of a species of *Nitrospira* bacteria that Hovanec discovered.

The product requires refrigeration to maintain its shelf life, as high temperatures can reduce the function of the living organisms over time.

Shelf life is a critical component of any pet retail product, according to Gary Jones, technical sales specialist at Aquarium Pharmaceuticals Inc. in Chalfont, Pa., which markets another bacteria product for aquariums.

"The hurdle biology has not been able to jump yet is to give nitrifying bacteria the stability they need on the retail shelf. A two- to three-year shelf life is what it takes to be a viable product in this industry," Jones said, adding that paying attention to refrigeration and shorter shelf life "would be a new mind-set for retailers."

But the results from BIO-Spira are worth the extra care required from retailers, Marineland said.

"What this means for the fishkeeping hobby is



BIO-Spira resulted from studies that identified bacteria responsible for detoxifying fish waste in aquariums.

huge," said Denny Adkins, vice president for corporate communications for The Aquaria Group, Marineland's parent company. "People can get through that critical early period of tank setup and keep their aquariums year in and year out. The impact is truly industry-wide."

"We estimate the bacteria market is about \$2 to \$3 million, but we expect it to grow considerably with BIO-Spira," he said.

Adkins said pre-cultured BIO-Wheels that contained the same bacteria species were used with success by Florida fish farmers who set up exhibition tanks at the recent Pet Industry Distributors Association show in Orlando, Fla.

Because many consumers don't understand the nitrogen cycle that takes place in a closed aquatic system, their new fish can die from ammonia or nitrite poisoning from fish waste, according to Loiselle, who teaches a course at the New York Aquarium on starting a home aquarium.

"The average freshwater hobbyist will give it two tries," Loiselle said. "If the second lot of fish is alive at the end of the month, they stay with it. If the second lot dies, the tank goes in the garage and they walk away from it as a hobby that's too difficult."

"BIO-Spira makes it more idiot-resistant. It should give people success as beginning aquarists."

Andrew Branson, curator of the Aquarium Department at the Tulsa Zoo in Oklahoma, also tested the product on a new freshwater aquarium. "It worked real well for us," Branson said.

"Normally the cycling process takes four to six weeks for the beneficial bacteria to develop on its own in the filters, if you don't seed it with gravel from another tank. We didn't seed this tank with anything, and we poured in the bacteria and added ammonia as a test. In eight days the ammonia levels were zero, which is much, much faster than you'd expect," he noted.

"There is the possibility we just got lucky, but if it does prove to be as good as it appears to be, it would definitely be exciting in the aquarium world," said Branson.

Hovanec obtained a U.S. patent on the *Nitrospira* bacterium after proving that it is novel and has utility. He was required to isolate and purify the bacteria and deposit it in a treaty bank, the American Type Tissue Culture bank, in Virginia.

Patents are pending on several nitrifying bacteria in Marineland's new BIO-Spira product, Hovanec said. He is also required under the patenting rules to store the bacteria deep-frozen at -78 degrees Celsius for 30 years.

Marineland Laboratories in Moorpark, Calif., contains the DNA freezer and an assortment of cutting-edge scientific equipment for microbial ecology and water chemistry research.

The *Nitrospira* bacteria are cultured and raised in the facility in eight towering 2,300-gallon tanks. Hovanec and his team of scientists and technicians carry out regular testing on the tanks to make sure the bacteria are growing well.

One type, species of *Nitrosospira* and *Nitrosomonas*, converts ammonia to nitrite, and another type, the *Nitrospira*, converts nitrite to relatively harmless nitrate within aquariums.

"It's easy to tell the difference," Hovanec told a visitor as he scooped murky-looking water from one of the tanks.

The ammonia-oxidizing bacteria in the water are a reddish orange, and the nitrite-oxidizing organisms are tan, he said.

Marineland's bacterial concoction has been

available to some commercial users since the beginning of this year, Adkins said. It has also been field tested at aquaculture facilities and public aquariums under its early designation as BIO-Balance, before the name BIO-Spira was selected.

Aquarium Pharmaceuticals' Jones remains skeptical about the new product.

"No one invented a new bacteria," he said. "Nitrifying bacteria have always been in aquariums, and *Nitrospira* is not the only beneficial bacteria in aquariums. To say this is the end-all bacteria is a big stretch," Jones said.

"Nitrifying bacteria have been bottled and shipped for years in the aquaculture industry, but the problem in the aquarium industry has always been with stabilizing the bacteria when the hobbyist uses it," he said.

But Hovanec said the aquarium industry has just been slow to accept new ideas.

"What we have done, and received a patent for, is the discovery of a new, novel bacteria --*Nitrospira* -- isolated from aquaria," said Hovanec.

"My research shows that the only nitriteoxidizing bacteria found in aquaria are members of the genus *Nitrospira*. *Nitrobacter*, which is what other companies sell and everyone assumed was the nitrifier, are simply not in aquaria. In addition, my research demonstrates that adding *Nitrobacter* to an aquarium does not result in its establishment.

"The quick take on my research is that we have discovered many new ammonia- and nitriteoxidizing bacteria and they, not the ones that we assumed, are the nitrifying bacteria critical to keeping our aquaria healthy," said Hovanec.

"In the aquatic microbial field, there's no debate anymore about the role of *Nitrospira* in the nitrogen cycle of a closed freshwater system," he said.

BIO-Spira will be featured at the World Wide Pet Supply Association show for pet retailers in Anaheim, Calif., in July, according to Marineland's Adkins.

"Independent pet retailers have the understanding and passion to give their customers advice. They want their customers to be successful at keeping fish," Adkins said.

> For more information www.marinelandlabs.com

Scientific Detective Work Led Hovanec to BIO-Spira



Dr. Tim Hovanec

For Tim Hovanec, an aquatic microbial ecologist, the discovery of the Nitrospira bacteria in aquariums was a happy accident that resulted from what he calls "evolving science," using sophisticated bacteria cloning and DNA sequencing techniques now available for routine scientific research.

The story of his discovery began 10 years ago when he was working to develop improvements to Marineland's BIO-Wheel, a wet/dry biological filtration system where beneficial nitrifying bacteria thrive.

For years, he said, scientists have assumed that the beneficial organisms that convert ammonialaden fish waste to relatively harmless nitrate in aquariums were the same Nitrobacter and Nitrosomonas bacteria found in soil. But when his tests repeatedly failed to find these bacteria in the closed aquatic system of aquariums, "My research took a big left turn," Hovanec said.

He turned to DNA cloning and sequencing to identify the bacteria responsible for detoxifying fish waste in aquariums.

What he found was a spiral-shaped bacterium of the phylum Nitrospira, and he published his results in a peer-reviewed scientific paper in the Journal of Applied and Environmental Microbiology in January 1998.

About the same time, three other teams of scientists in Australia and Germany discovered the same nitrifying bacteria at work in a wastewater treatment plant and a sewage plant, and several more scientific papers were published in the same microbiology journal.

"These bacteria are the real stuff," Hovanec said. "My research has been corroborated and it's been highly scrutinized."

Experts Give BIO-Spira Thumbs Up!

Over the past 18-months, BIO-Spira has been in use at many world-leading public aquariums and aquaculture facilities. The results speak for themselves.

"BIO-Spira lives up to its claim. Marineland Labs has a winner. I have no hesitation whatsoever in recommending it to novice and experienced hobbyists alike."

Paul M. Loiselle, Ph.D. Curator of Fishes, New York Aquarium

"BIO-Spira dramatically shortened the time it would have taken to get the filtration matured and fishes on exhibit."

Andrew Branson, Curator Tulsa Zoo and Living Museum "BIO-Spira is the best nitrifying product we have ever come across! None of the competing products even comes close."

> Lars Olsen Aquafuture Corporation

"We tried BIO-Spira on several different freshwater systems with excellent results. Non-toxic nitrogen levels were established immediately."

Mike Brittsan, M.Sc., Curator Columbus Zoo and Aquarium

