WORKSHOP

TargetFish - Targeted disease prophylaxis in European fish farming

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Introduction

TargetFish is a large collaborative project funded by the European Commission under the 7th Framework Programme for Research and Technological Development (FP7) of the European Union (Grant Agreement 311993). The project that will run for 5 years, started in November 2012 and thus is approximately halfway. TargetFish brings together a large number of leading European research groups (RTD) that are experts on fish pathology and immunology and small-to-medium enterprises (SME) as well as some larger industries from the Biotech and Veterinary sectors, which all share a common interest and experience: vaccination of fish.

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TargetFish is revolutionary with respect to its aims to i) not only generate fundamental knowledge for the development of next generation vaccines and different routes of vaccine administration, but also ii) validate this knowledge by actively working on a rapid implementation of improved, or new prototype vaccines.

Industrial Workshop

TargetFish has the ambition to demonstrate market applicability of a selected number of improved, or new prototype vaccines. Via frequent joint meetings of all its partners, be it research group or enterprise, with representatives of nutrition and vaccine industry in an Industry Forum, TargetFish aims to drive prototype development in an industrially applicable way. This will facilitate adoption of new intellectual property and stimulate the presentation of new fish vaccines on the market.

To achieve its challenging tasks, TargetFish unites 29 partners from 10 EU member states, 2 associated countries and 1 International Cooperation Partner Country (Chile). The large multidisciplinary consortium consists of an approximate equal number of RTD and SME partners who cooperate closely. The results thereof are communicated to those people and organisations who address the fish vaccine market, and are interested in TargetFish, via a monthly newsletter and via Industrial Workshops. After a first meeting during the 16th international EAFP conference organised in Tampere, Finland, two years ago, the EAFP meeting in Las Palmas set the stage for the second TargetFish Industrial Workshop. The present workshop report provides a short overview of TargetFish highlights presented to, and discussed with, those present at the 17th international EAFP conference and dealing with fish vaccine development, policies and laws, production and delivery.

Funding by the European Union

The aim of TargetFish is to advance the development of existing (but sometimes insufficient or suboptimal) and new prototype vaccines against a number of socio-economically important viral or bacterial pathogens of Atlantic salmon, rainbow trout, common carp, sea bass, seabream and turbot. Parasites are not a subject of TargetFish, but of another large collaborative project funded by the European Commission via the Horizon 2020 Programme; ParaFishControl. TargetFish builds on a generic knowledge- and technology-base for the fish immune system provided by the FP6-funded project Imaquanim, allowing for rapid progress on identifying key elements that determine adaptive immunity and memory, creating a scientific base for successful vaccination and long-term protection of fish.

TargetFish aims to provide a long-lasting contribution to the prevention of important fish diseases in the European aquaculture industry. Thereby, as has been shown in Norway during the last 20 years upon the implementation of efficient fish vaccines, we expect to reduce impact of veterinary treatments on the environment. The international and multidisciplinary team of scientists that form TargetFish is well equipped to achieve the continued training scientists at research institutes, universities, fish farms as well as nutrition and vaccine industry.

TargetFish

Traditionally, many fish vaccines have been based on inactivated bacteria or viruses, and several of these have proven to be extremely (cost) effective. But not all fish pathogens can

be fought with these type of vaccines and sometimes next generation subunit vaccines based on specific antigens are needed. However, these recombinant vaccines sometimes have lower immunogenicity than complete pathogens, thus specific adjuvants must be combined with the antigen to reach significant protection levels. On the other hand, DNA vaccines, but also live attenuated vaccines, can be highly efficacious, although safety concerns limit their use. DNA vaccines administer a DNA sequence coding for the target antigen: the DNA is injected in the host, transcribed and the protein produced by the host itself. TargetFish not only studies new antigens but also new formulations of DNA plasmids to optimize their application and to provide safety and efficacy data to facilitate future decision-making on highly-effective DNA vaccines. Finally, most fish vaccines in the market are delivered by injection, this being a labour-intensive, expensive method that provokes stress in the fish. Mucosal vaccination routes (immersion or oral) would certainly be more desirable, but their optimization still requires more research. TargetFish also focuses on investigating mucosal vaccination routes and associated immunity and in developing antigens and adjuvants specifically suited for mucosal delivery.

Antigens for vaccine development

Antigens can be produced by traditional methods, including inactivation of bacteria and viruses, or produced in bulk culture for example when expressed as recombinant products in a bacterial or yeast expression systems. The yeast *Pichia pastoris* has a high growth rate and can grow on a simple, inexpensive medium in either shake flasks or a fermenter, making it suitable for both small- and large-scale production of antigens for fish vaccines. Under optimal conditions, Pichia can grow to very high cell densities, and produce recombinant proteins with the required disulfide bonds and glycosylations patterns, at very high protein yields. At the Industrial Workshop, progress made by TargetFish on developing a prototype Pichia-based vaccine against viral encephalopathy and retinopathy virus (VERV) and infectious pancreatic necrosis virus (IPNV) was discussed.

Rainbow trout fry syndrome (RTFS) is caused by gram-negative *Flavobacterium psychrophilum* (Fp) bacteria, can affect all species of salmonid fish and result in high mortalities, especially in young fish. During the Industrial Workshop, progress was discussed on a prototype polyvalent vaccine based on water in oil emulsions of multiple Fp bacterial strains, which were shown to provide good protection against experimental challenge.

Vaccine delivery

Injection vaccination appears the most effective way of vaccinating fish, at least with respect to levels of protection. Since injection by hand is extremely time consuming, automatic vaccinating machines may help achieve mass injection vaccination. Of extreme importance is that such machines are designed properly to fit the fish to the machine correctly, so that injections are placed correctly in the intraperitoneal cavity. Minimal size requirements for the to-be-injected fish have been a limiting factor for the application of automatic vaccinating machines. During the Industrial Workshop, progress was discussed on the development of automatic vaccinating machines for injecting small turbot or sea bass of 5-10 gram only, allowing for vaccination of juvenile fish in the near future.

Mucosal delivery systems

Microencapsulation is the coating of substances of interest (here; vaccine antigens) with specially selected materials (here; alginates) in order to modify/incorporate properties that are not inherent to these materials. Among the most important objectives to be achieved with microencapsulation are protection and stabilisation of the vaccine antigens with respect to the environment and/or during storage, until the vaccine antigens reach the site of action (here; the local immune system in the fish' gut). Mass delivery systems using biodegradable and biocompatible materials such as alginates for inclusion in oral vaccines are highly promising: the loading capacity of alginate particles can be high, as well as the stability and preservation of the encapsulated proteins. Alginates are considered safe and can provide controlled-release of encapsulated protein to target antigen delivery to antigen-presenting cells of the local immune system. Alginates are natural, biodegradable and muco-adhesive and can help reduce degradation in the acidic environment of the stomach or intestine. During the Industrial Workshop, progress was discussed on the use of alginate microencapsulation of antigens from important fish bacteria and/or viruses, including VERV. The size of the particles should be proportionate to the loaded protein and administration route and thus the interaction between alginate particles and exact vaccine antigens determine the final product.

Protective immunity

TargetFish is addressing different delivery methods for fish vaccines, with automatic vaccination but also oral delivery as promising methods for mass vaccination. Vaccine antigens often require protection against the harsh environment of the stomach and/or intestine and alginates may help achieve this protection in a biologically safe manner. Water-in-oil emulsions of antigen and adjuvant often work efficiently, especially in the case of injection vaccines, but can sometimes trigger local inflammation and may not provide the optimal formulation for oral vaccines, eg those delivered by alginates to the fish mucosa. Memory and specific immunity are central to successful vaccination of fish. Now that three antibody types have been identified in fish; immunoglobulin (Ig)M, IgD and IgT, measuring specific IgT in the mucosa of fish could help provide a measurement of local (mucosal) immune responses after oral or immersion vaccination. TargetFish will continue to develop fundamental knowledge for next generation fish vaccines and for application of different routes of vaccine administration, while validating new knowledge by actively involving the industry. The partners of TargetFish look forward to present and discuss their results in 2017 during a next Industrial Workshop at the EAFP in Belfast, Ireland.