

Scale-up of vaccine production in a microalgal host for animal trials

A BioProNET business interaction voucher has enabled Saul Puron and Brenda Parker from University College London to work with start-up company MicrosymbiotiX to investigate the potential of microalgae as an oral delivery system for vaccinating farmed fish.

Previous work established that the chloroplast of the microalga *Chlamydomonas reinhardtii* could be engineered to express a recombinant subunit vaccine against a major viral pathogen of fish.

The aim of this project was to scale-up the phototrophic production of *C. reinhardtii* and optimize the downstream processing of the algae to produce sufficient dried biomass for formulation into fish feed that will then be used in viral-challenge trials.

The work carried out was as follows:

- 1. Optimisation of *C. reinhardtii* engineered to produce a subunit vaccine against the virus:** Using a simple photobioreactor system based on single-use polythene hanging bags, growth rates, algal biomass yield and antigen yield were evaluated under either batch or continuous cultivation and under different media and light regimes.
- 2. Evaluation of biomass harvesting methods:** Yield and integrity of the algal cells following either flocculation with chitosan or disc-stack centrifugation was compared.
- 3. Evaluation of vaccine stability:** The stability of the protein in the dried biomass at room temperature was explored by western blot analysis.

The combination of these three steps resulted in an optimised production regime for algal biomass containing the vaccine

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4. Towards fish feeding trials: 50g of dried algal powder from three *C. reinhardtii* strains (negative control, vaccine-expressing strain and a GFP-expressing strain) were produced through an agreement with the algal cultivation company, A4F (Portugal) and sent to the Center for Aquaculture Technologies (California, USA) for fish feeding and challenge trials.

Next steps

Doses of the algae (100 µg antigen per dose) have been given to test fish. The blood sera of these fish are being examined for antibodies to the antigen by MicrosymbiotiX, prior to carrying out disease challenge trials.

Key outcomes:

The project has significantly advanced our understanding of scale-up and downstream processing of engineered algal strains

The availability of validated algal biomass has allowed the company to move forward quickly by instigating the fish trials, and has allowed the company to secure further funding

Two publications on the underpinning research carried out in the project are in progress (one manuscript in review, one in preparation)

The academic partners have used this research to secure a NERC/BBSRC grant on the use of algal-based vaccines in aquaculture: BB/5004327/1

