

During the past three decades, European shellfish aquaculture has seen a continuous expansion while providing employment to communities often located in remote coastal areas. Although shellfish contribute to the improvement of water quality by filter-feeding microscopic particles present in the water, they can accumulate faecal material from human or animal sources as well as potent algal toxins produced by harmful algal blooms. A ban on shellfish harvesting in areas affected by those microbiological (bacterial and viral) and toxic events can adversely impact the financial viability of the affected shellfish farm particularly when closures are prolonged and recurring.

To mitigate the negative impact caused to shellfish by those contamination events and also to tackle a parasitic disease (bonamiasis) specifically affecting the native oyster *Ostrea edulis*, the BEADS project (Bio-engineered micro Encapsulation of Active agents Delivered to Shellfish) was specifically set up to address these challenging issues by seeking to boost shellfish immune response and improve purification efficiency by means of active agents delivered directly to the bivalves through microencapsulation.

The project demonstrated that digestible alginate microcapsules fed to various shellfish species were effectively ingested, and it also found the ingestion rate was linked to the size of the microcapsules, the smaller ones being ingested at a faster rate. Those experiments demonstrated the microcapsule technology was an ingenious tool to introduce biological agents such as bacteria into shellfish.

A large number of bacteria (more than 500) were isolated during the course of this project. Some bacteria were isolated from shellfish contaminated with okadaic acid (OA) and domoic acid (DA) and a number of isolates (32) were selected after in vitro toxin degradation experiments shown increased bacterial growth (at least 40%) in the presence of OA and DA over 72 hours. Those 32 isolates were characterised using molecular sequencing and the results revealed a wide variety of bacteria species.

Further toxin utilisation experiments carried out at 12°C and 20°C and monitored by liquid chromatography-tandem mass spectrometry (LC-MS/MS) led to the selection of 4 bacterial isolates with promising in-vitro toxin degradation ability (toxin degradation higher than 20%). These bacteria were microencapsulated and were used in laboratory feeding trials. The results of the trials carried out on king scallops and blue mussels at 12°C did not overall improve the detoxification rate in the tested shellfish over a 7 days period. These experiments have highlighted the necessity of finding fast acting detoxifying bacteria to limit the confinement of shellfish in holding tanks to avoid stress related shellfish losses.

A large number of bacteria were also screened for antimicrobial activity against a selection of pathogenic bacteria and viruses. One bacterial isolate (lactic acid bacterium) which was identified after DNA sequencing showed antilisterial activity against *Listeria monocytogenes* CECT 935 and several strains of *Listeria monocytogenes* isolated from seafood products. The active substance (*E. hirae* 3M21) was partially characterised and shown to be proteic with a molecular weight of 13.5 kDa. Survival rates of free and encapsulated *E. hirae* 3M21 were subsequently assessed in seawater at 15°C and in digestive gland fluids from mussels and oysters. Results showed an absence of viability loss in any of the cases studies. Survival of encapsulated 3M21 in saline buffer was 99% after 70 days in refrigerated storage (4°C) without any observed loss of antilisterial activity. Finally, the antilisterial isolate 3M21 was microencapsulated and fed to shellfish. Results showed higher bacterial counts ($p < 0.05$) detected in the shellfish digestive glands while antilisterial activity was also confirmed thus validating the utilisation of the microencapsulation technique.

Feeding experiments assessing the ability of *Ostrea edulis* to ingest alginate microcapsules containing some fluorescent microbeads revealed the oyster's blood cells had actively taken up the microbeads. Screening also revealed the presence of fluorescent microbeads within the digestive and connective tissue areas known to be infected by *Bonamia ostreae*, thus validating the use of microencapsulation as a potential tool for the transport of immunostimulants directly to the infected areas. An experiment involving the microencapsulation of candidate immunostimulants was carried out to determine if those active compounds could stimulate the native oysters' immune system. It was found that a particular dose of each immunostimulants evoked a response in both cellular and non-cellular parts of the blood of the oysters. In a second set of trials using two additional immunostimulants, it was found the onset of oysters' mortalities had been reduced as well as the impact of the disease. In addition, there was a reduction in the prevalence of infection during the trial not related to mortalities indicating that the infection had been eliminated. The blood cells of the *O. edulis* had been stimulated resulting in better ability to fight the *Bonamia* infection.