Characterization of abalone *Haliotis tuberculata*-*Vibrio harveyi* interactions in gill primary cultures

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The decline of European abalone *Haliotis tuberculata* populations has been associated with various parasites among them the bacteria of the genus *Vibrio*. Following the summer mortalities of 1998 and 2000 in France, *Vibrio harveyi* strains were isolated from both farmed and wild abalones, allowing *in vivo* and *in vitro* studies on the interactions between abalone *H. tuberculata* and *Vibrio harveyi*.

This work reports the development of primary cell culture from abalone gill tissue, a target tissue for bacterial infection, and their use for *in vitro* study of abalone cell – *Vibrio harveyi* interactions. Gill cells originated from four-day-old explant primary cultures were sub-cultured in multi-well plates and cultured for up to 24 days. The characterization of cell types and the physiological status of cultured cells were monitored over the time in culture. Results showed that primary cell cultures from abalone gills are suitable for *in vitro* study of host-pathogen interactions, and provide complementary assays to *in vivo* experiments.

Then, gill cell cultures were used to investigate *in vitro* the mode of action of *V. harveyi*. We have investigated the effects of two different bacterial strains on gill cells : a pathogen bacterial strain ORM4 which is responsible of abalone mortalities and LMG7890 a non-pathogenic strain. Cellular responses in contact of different concentrations of bacterial strains were evaluated by measuring mitochondrial activity (XTT assay) and phenoloxydase activity, an enzyme which is strongly involved in pathogen response. The ability of gill cells to phagocyte *V. harveyi* was studied using flow cytometry and observed in fluorescent microscopy with GFP-tagged bacteria.

The gill cells of abalone present the ability of phagocyte 70% of pathogen and non-pathogen bacterial strains after two hours of contact by using flow cytometry. Moreover by fluorescent microscopy GFP-tagged bacteria were shown to be internalized by target cells. During phagocytosis process we evidenced that *Vibrio harveyi* bacteria induced significant changes in gill cells metabolism and immune response.