Spanish National project: BIVALGEN
Responsable scientist: Dr. Beatriz Novoa
Period: 1/12/ 2003 - 1/12/2006

Objectives:

1. Evaluation of the effect of immunostimulants on the immune response of bivalves (mussel *Mytilus galloprovincialis* and clam *Ruditapes decussatus*). Effect of immunomodulation on the resistance against pathogens (two vibrios associated to clam mortalities)
2. Characterization of genes involved in bivalves immune responses by two different strategies:
    a. Homology RT-PCR. Using primers designed in conserved sequences of cytokines
    b. Libraries substraction in a selected model. Trying to identify differentially expressed ESTs that are transcibed in treated animal but not in controls.

Several experimental infections were performed both in vivo and in vitro. Hemolymph samples were collected after different time points.
Several immunological parameters were studied:

Viability
Phagocitosis
Respiratory burst activity
Release od nitric oxide.

Results:

1. Several substances such as zymosan, β-glucans, LPS, dead and live, Poly I:C or PMA were assayed in roder to analyzed their effects on the bivalve immune responses
NO production increased significantly in β-glucans treated mussels and clams. In mussels, β-glucans increased by themselves the release of free oxygen radicals and also were able to enhance the phorbol 12-myristate 13-acetate (PMA) effect on this hemocyte activity. However, high doses of β-glucans when combined with zymosan decreased this respiratory burst. In clams, hemolymph treated with several doses of β-glucans detained the growth of the three bacteria (*Vibrio algynolyticus* (strain TA15), *Vibrio splendidus* (strain TA2) and *Escherichia* coli (strain ATCC 13706)) studied.

2.RT-PCR: Using primers designed in conserved sequences of cytokies or facterors involved in innate immune responses, we were able to amplify some products possibly related to immune moleculaes. A collection of about 50 ESTs were obtained. However, this method resulted to be not very succesful due to the low homology present in the bivalves proteins.

SSH: We were able to conduct the SSH technique in clams infected with dead bacteria. A collection of 258 ESts were obtained, which were more expressed in treated animals with respect to controls.