Study of interactions between flat oyster, Ostrea edulis, and the parasite Bonamia ostreae using flow cytometry and suppression subtractive hybridization (SSH).

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Infection with *Bonamia ostreae* - Notifiable to the OIE and EU. - This parasite is affiliated with Haplosporidians (Cochennec et al. 2000, Carnegie et al. 2000) and belongs to the phylum of cercozoan (Cavalier-Smith & Chao 2003). - Natural host is the European flat oyster Ostrea edulis. - Target cells: haemocytes.
Relationships between host and pathogen.

- Direct transmission possible
- Prepatent period 3-5 months
- Mortality preferably on 2-year-old oysters
- Resistance (natural / genetic selection)
- Parasite is engulfed and not destroyed after infection in vitro.

Life Cycle? Resistance?
Objectives

1- Study of haemocyte activities in the presence of Bonamia ostreae by flow cytometry.

2- Identification of genes expressed during an infection by SSH.
Material for Flow cytometry and SSH

- **Animals and hemolymph collection:**
  - 2-years-old flat oysters Ostrea edulis were collected from Bay of Quiberon (endemic area).
  - Hemolymph was withdrawn from the adductor muscle and filtered at 75µm. The hemolymph was pooled.

- **Parasites:**
  - Bonamia ostreae was obtained by purification from highly infected oyster according to Mialhe et al. (1988).
  - Inactivation of parasite was done at 100°C during 15 minutes.
Flow cytometry - Method

Haemocytes  Haemocytes + live parasites  Haemocytes + inactive parasites

Ratio 1/5 and 1/10

Cell labelling with specific fluorochrome:
- Propidium iodide (mortality)
- Fluorodiamine (esterase activity)
- DCFH DA (free radicals)
- Beads 1 μm (phagocytosis)

Analyses performed using flow cytometer Beckman Coulter EPIC XL 4

2h contact
Flow cytometry - Methods

- Interpretation of results:

1- population of negative cells.
2- population of moderately stained cells.
3- population of strongly stained cells.
Flow cytometry - Results

Esterase activities decrease in the presence of both live and inactive parasites.
Free radical activities decrease in the presence of both live and inactive parasites.
Suppression subtractive hybridization (SSH)-Methods

Haemocytes + live parasites

Extract total RNA by Trizol reagent (invitrogen)

Haemocytes

Translate total RNA in cDNA (using the subtraction kit Clontech)

2h contact
Suppression subtractive hybridization (SSH) - Results

- 1104 clones were selected.
- 391 clones showed a differential expression and were sequenced.

Sequences analysed using BLASTX (Altschul et al. 1997) in the database NCBI.
- 96 sequences (25%) presented a significant homology (E value < 10^-4).
Suppression subtractive hybridization (SSH) - Results

Distribution of the genes according to the cluster of orthologous genes

- Detoxification stress protein (4%)
- Metabolism (11%)
- Ribosomal (18%)
- Hypothetical protein (18%)
- Cell cycle, DNA repair, protein regulation and transcription (18%)
- Respiratory chain (11%)
- Cytoskeleton
- Cells communication
Suppression subtractive hybridization (SSH)- Results

- Candidate genes expressed by the parasite: 36%.
  - Actin, Heat shock protein 90, Cofilin, Alpha tubulin

- Candidate genes expressed by the host: 54%.
  - MAPK organizer 1, lipoprotein receptor-related protein 6, actin bundling protein

- 10% are not associated with parasite or host.
Conclusions

• **Flow cytometry:**
  - Parasites (live and inactivated) induce modifications of haemocyte activities: a decrease of esterase activities and an inhibition of free radical.

  - Inhibition of free radical was also reported for other intracellular parasites like *Toxoplasma gondii* (Shrestha, 2006).
Conclusions

- Bonamia ostreae seems to block defence mechanisms including oxidative burst. This capacity probably helps its established inside the host cell.

- Bead phagocytosis is unchanged in presence of parasite compared with haemocytes alone.
Conclusions

- **SSH:**
  - Identification of candidate genes belonging to the parasite and to the host.
  - No identification of genes involved in the immune response or of cell detoxification. These results confirm those obtained by flow cytometry.
  - Presence of genes related to the cytoskeleton (actin, myosin, alpha tubulin, actin binding and cofilin).
  - The cytoskeleton plays a key role in the invasion of the host by *apicomplexans* (Naomi.S et al 2002).
Perspectives

• Flow cytometry results will be completed by a study of haemocyte activities at different times.
• SSH results motivate studies on cytoskeleton modifications using flow cytometry.
Perspectives

• Expression at different times of selected genes would allow to understand these interactions.

• It would be interesting to complete these results by study of interaction between parasite and haemocytes:
  - from Ostrea edulis resistant strain.
  - from Crassostrea gigas, an oyster species not susceptible to Bonamiosis.
Thanks for my collaborators.

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and Nicole Faury, Béatrice Gagnaire, Bruno Chollet.
Thanks for attention.