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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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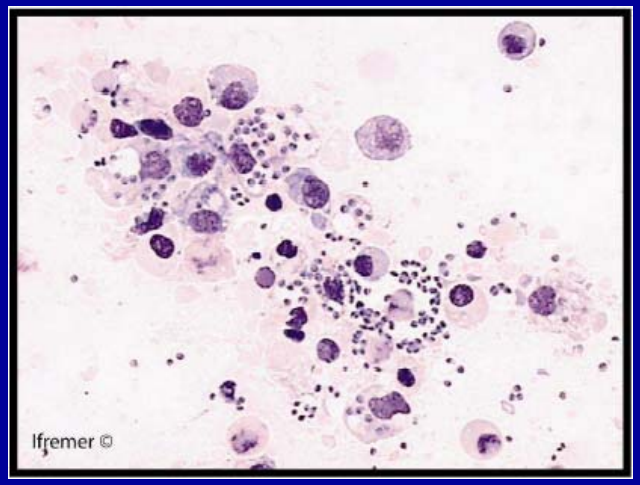

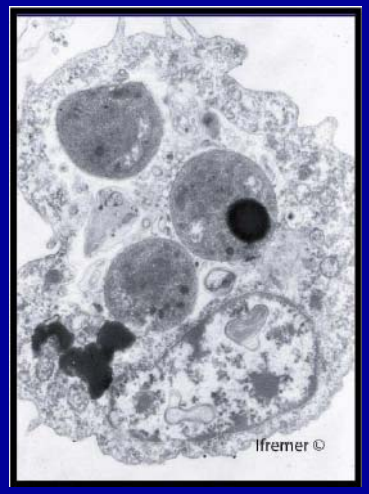

EUROPEAN ASSOCIATION OF FISH PATHOLOGISTS
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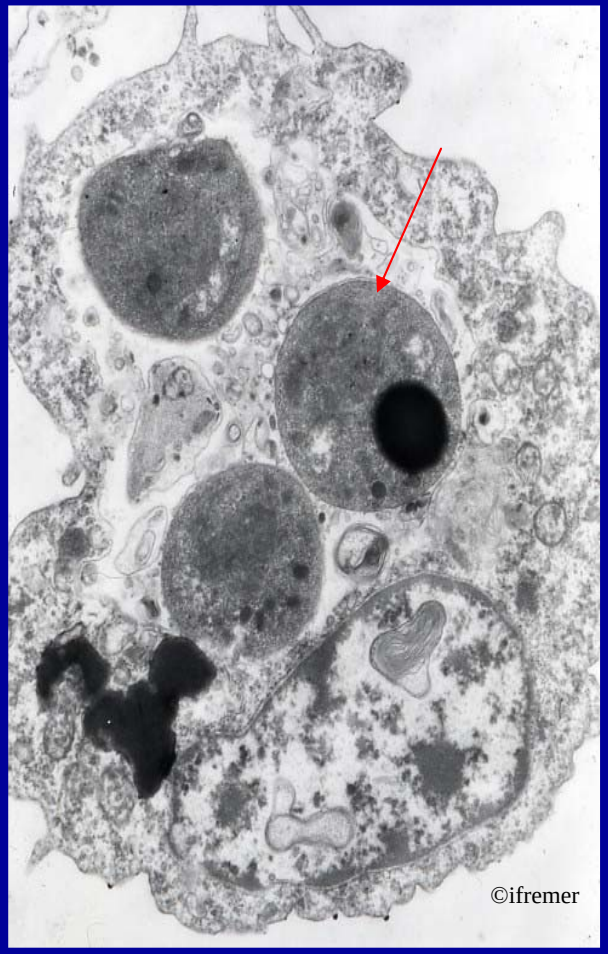
"Diseases of Fish and Shellfish"
16th – 20th September, 2007 - Grado, Italy

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.

Life Cycle?
Resistance?

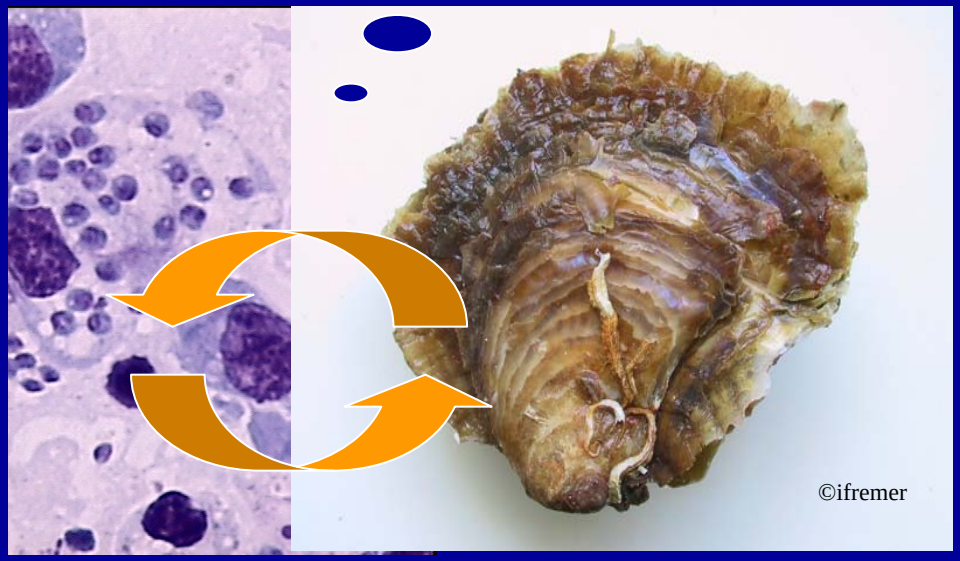
-Direct transmission possible

-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*.



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).

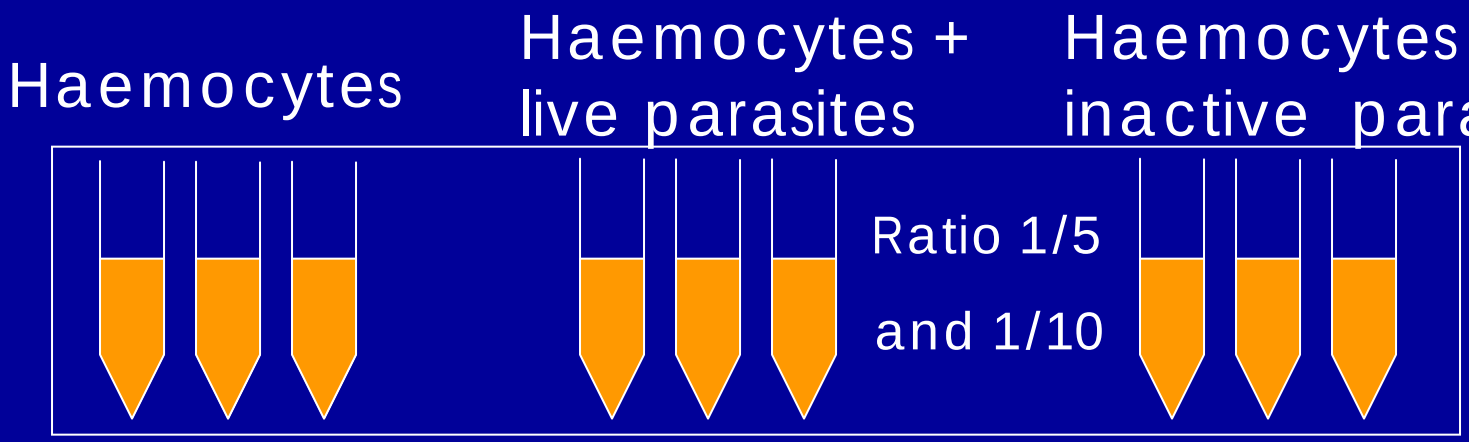
  -Hemolymph was withdrawn from the adductor muscle and filtered at 75µm. The hemolymph was pooled.

- 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



2h contact

Cell labelling with specific fluorochrome:

-Propidium iodide (mortality)

-Fluorodiamine (esterase activity)

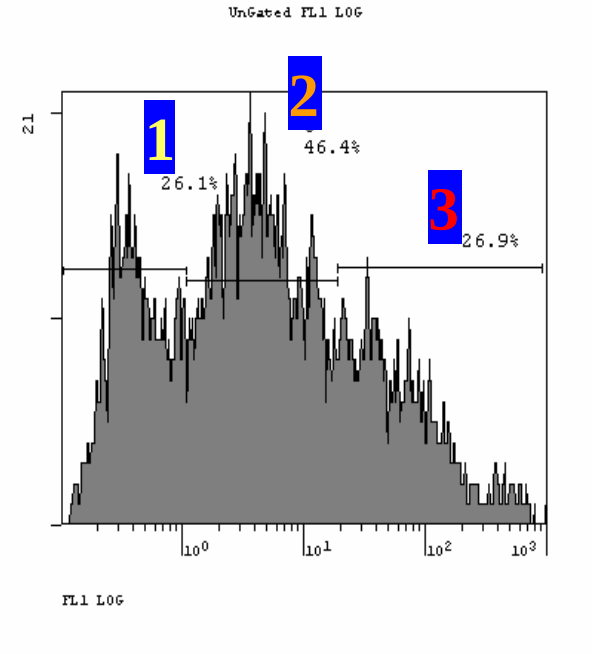
-DCFH DA (free radicals)

-Beads 1 µm (phagocytosis)

Analyses performed using flow cytometer Beckman Coulter EPIC XL 4

Flow cytometry- Methods

-Interpretation of results:

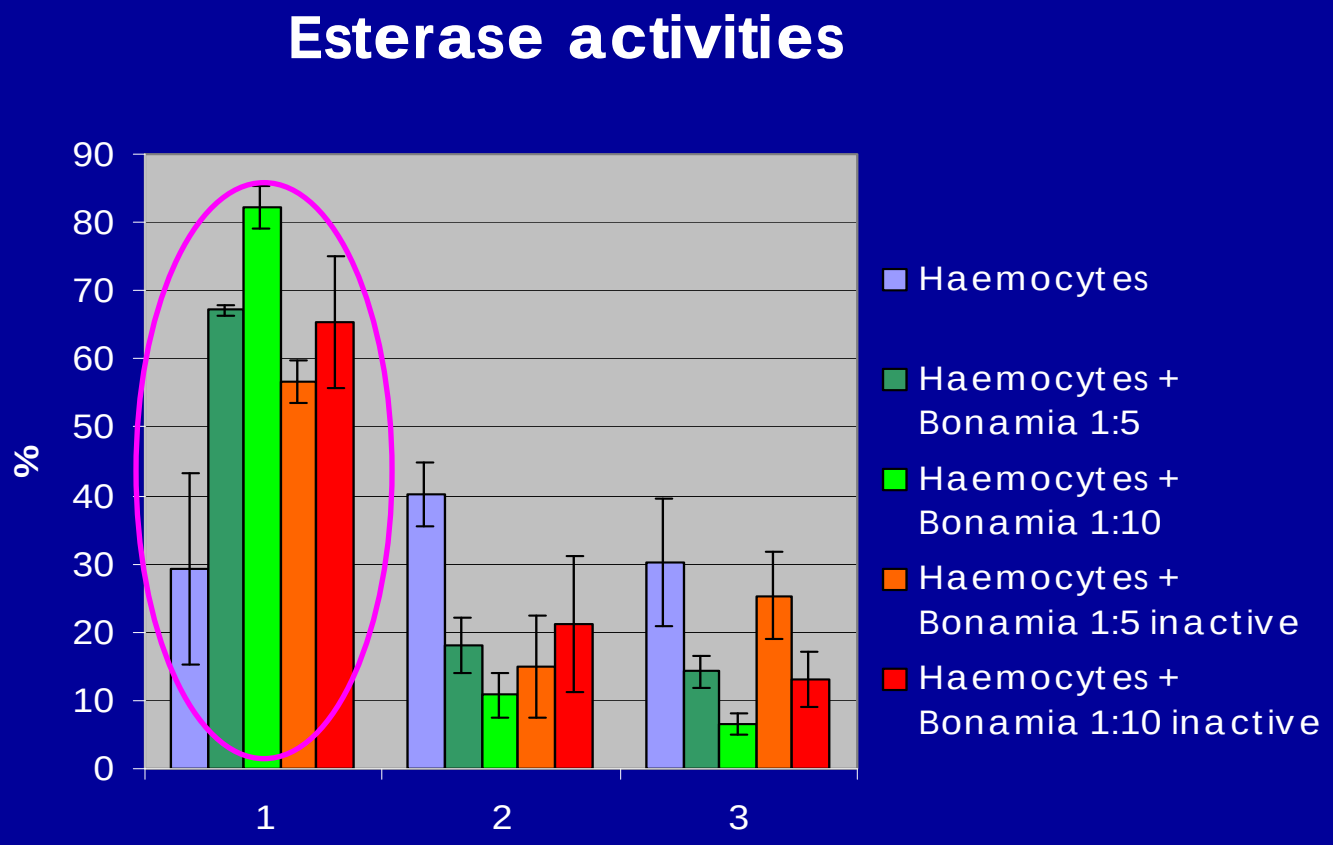


1- population of negative cells.

2- population of moderately stained cells.

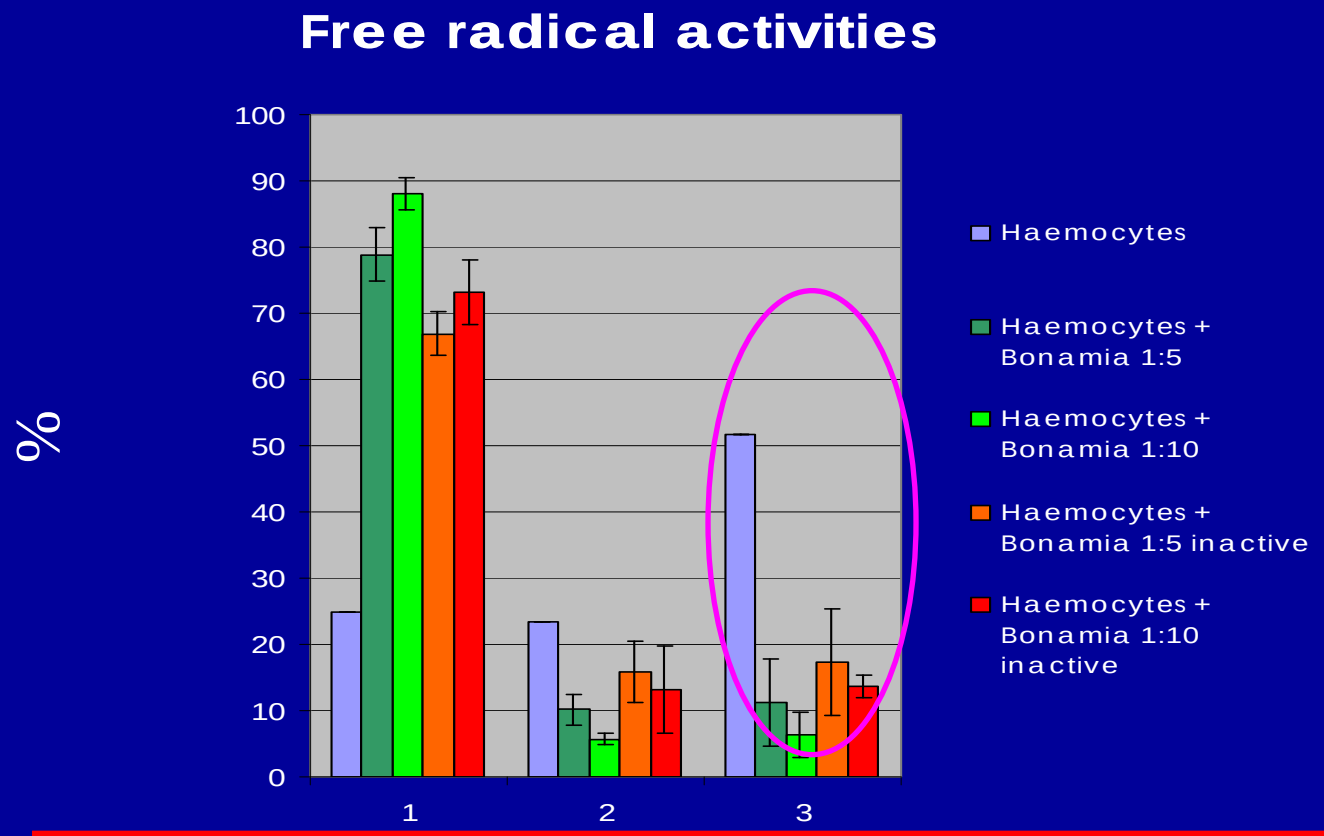
3-population of strongly stained cells.

Flow cytometry- Results



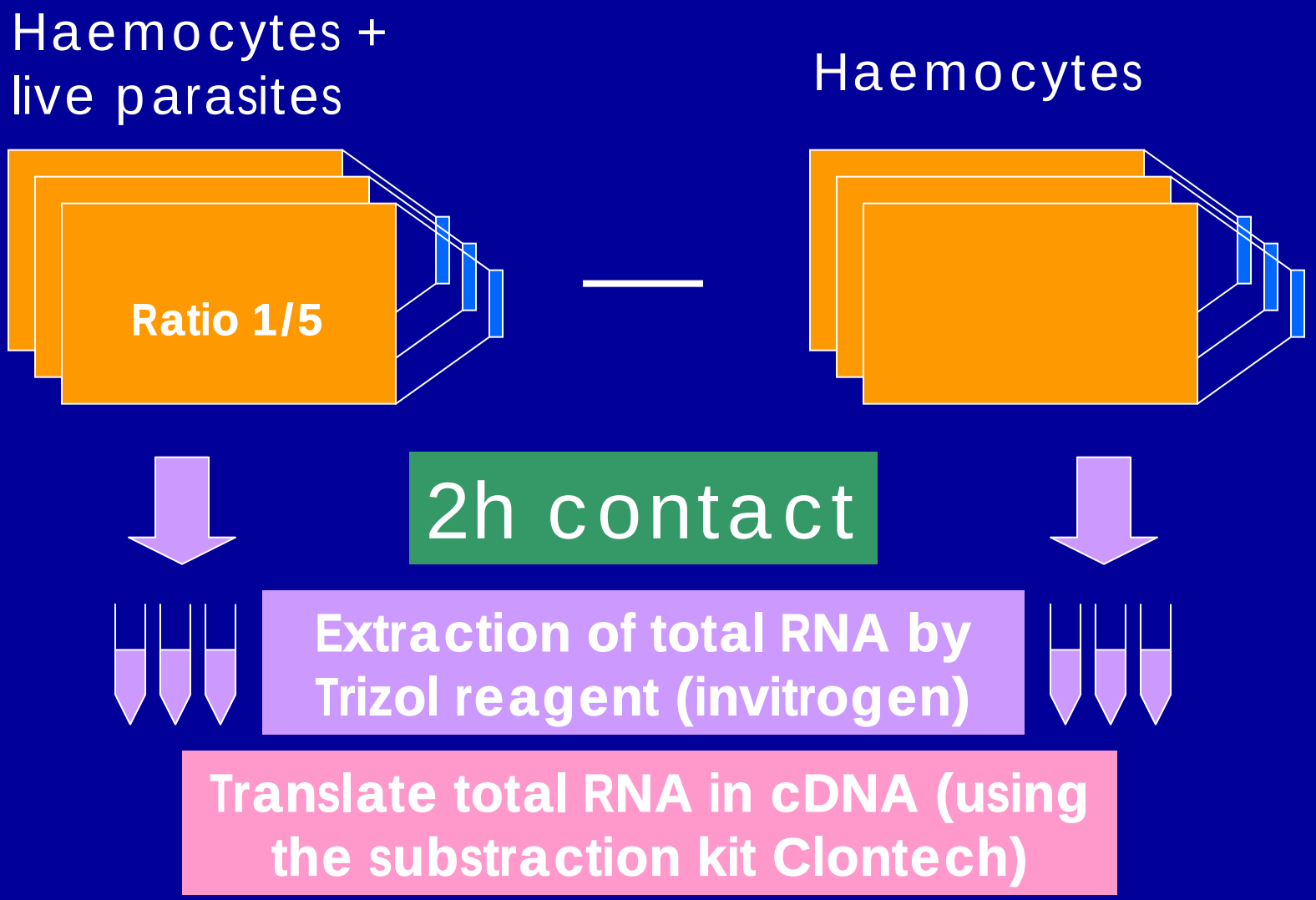
Esterase activities decrease in the presence of both live and inactive parasites.

Flow cytometry- Results



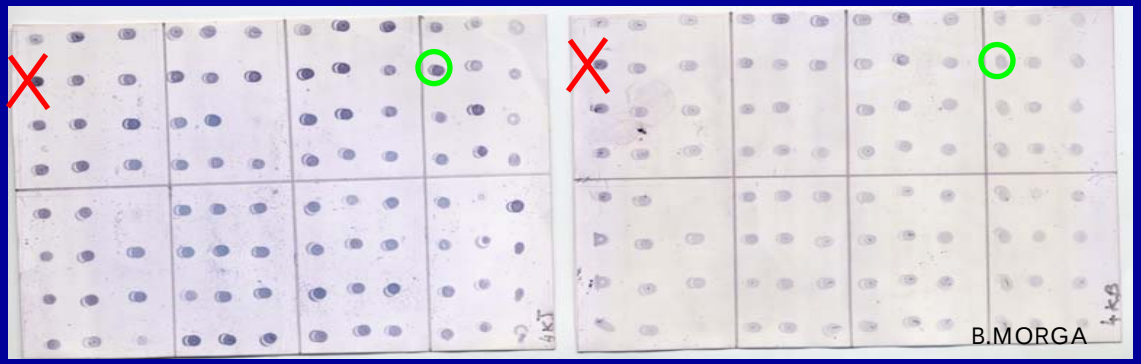
Free radical production decrease in the presence of both live and inactive parasites.

Suppression subtractive hybridization (SSH)-Methods



Suppression subtractive hybridization (SSH)- Results

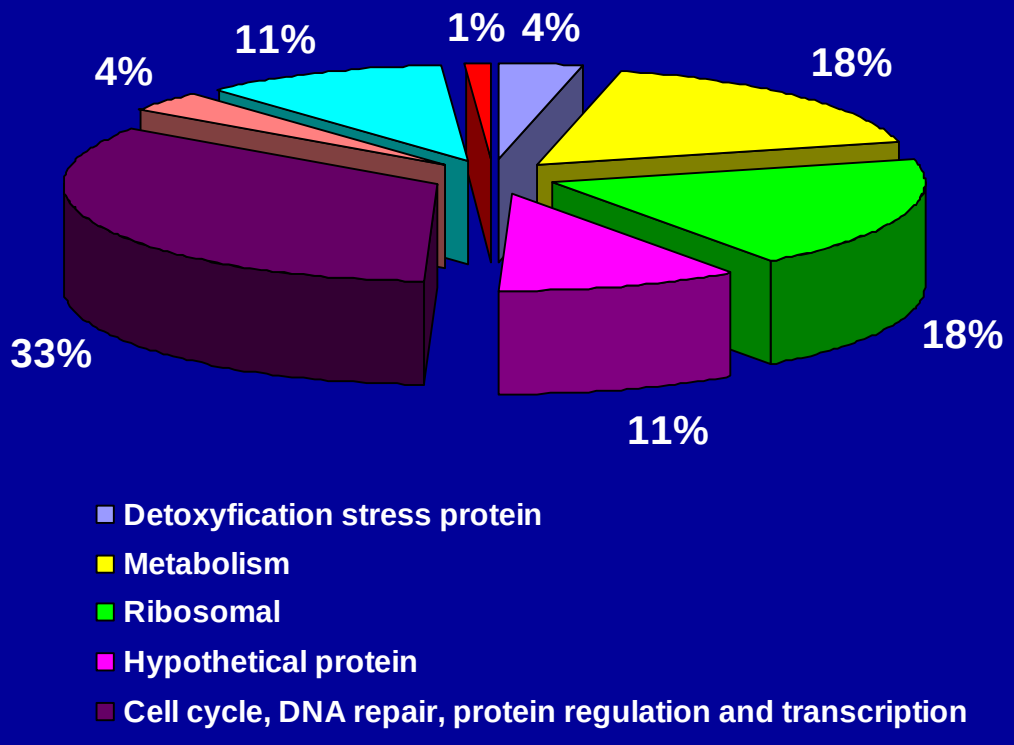
- 1104 clones were selected.
- 391 clones showed a differential expression and were sequenced.



- 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 hybrizidation (SSH)- Results

Distribution of the genes according to the cluster of orthologous genes



Suppression subtractive hybrizidation (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 oxydative 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 these 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 suceptible to Bonamiosis.

Thanks for my collaborators.

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Co-director PHD: Isabelle ARZUL

and Nicole FAURY, Béatrice GAGNAIRE, Bruno CHOLLET.

Thanks for attention.