GENOME-WIDE ANALYSIS OF DNA METHYLATION OF ATLANTIC SALMON IN RESPONSE TO STRESS

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DNA methylation

DNA methylation is one of the most commonly occurring epigenetic events taking place in the genome. The alternative most evaluated correspond to CG – C(CH3)G.

This change, though heritable, is reversible, making it a therapeutic target.

DNA methylation is a covalent modification of DNA that does not change the DNA sequence, but has an influence on gene activity.

Methylation modifications:

Cytosine

5-Methylcytosine
○ Over 70% of DNA from mammalian somatic tissues is methylated at 70% of all CpG sites (e.g., satellite DNAs, repetitive elements including transposons, nonrepetitive intergeneic DNA, and exons of genes.

○ Key exceptions of this global methylation of the mammalian genomes are the CpG islands (regions with high CpG density). Most CpG islands marks the promoters and 5’ domains of genes. Approximately 60% of human genes have CpG island promoters.
What Does “Epigenetics” Mean?

Epigenetics describes phenomenon in which genetically identical cells or organisms express their genomes differently, causing phenotypic differences.
STRESSORS AND STRESS IN SALMON

- Physiological/psychological stress
- Toxicological stress
- Environmental stress
- Handling, harvesting, stocking, etc.
- Immunological stress
- Nutritional stress
- Social stress
- Endocrine adaptation
- Behavioral adaptation
- Antioxidative proteins
- Immunological adaptation
- Metabolic adaptation
- Detoxification process
- Anti apoptotic process
- Cortisol process
- Cytoskeletal changes
- Unfolded protein (HSP)
- Changes gene expression
- Dysfunctional metabolic/mitochondrial
- Apoptosis
- Necrosis
- ROS production
- Immunosuppression
- Bebavioral maladaptation
- Distress
Epigenetic gene regulations: Two major mechanisms

- DNA Methylation
- Histone modification
MATERIALES AND METHODS

STEP 1. DIFFERENTIATION OF UN FROM METHYLATED DNA. GENOME-WIDE

Double enzyme combinations:
• EcoRI/MspI and EcoRI/HpaII, used to digest the DNA samples

PCR is used to selectively amplify the DNA fragments

STEP 2 DIFFERENTIATION UN FROM METHYLATED DNA

• MICROARRAYS/BEADCHIPS
• NEXT GENERATION SEQUENCING
• METHYLATION-SENSITIVE AMPLIFIED POLYMORPHISM (MSAP)
**MATERIALES AND METHODS**

**STRESS CHALLENGE MODEL:** handling and confinement experimental stress

Pure farmed Atlantic salmon strains, Gaspe and Laks (Marine Harvest) unselected, mixed-sex; mean mass ± SEM: 62.18 ± 1.6 g; mean length ± SEM: 17.64 ± 0.13

**Control**
- After adaptation, 10 fishes were sampled and immediately euthanized

**Step 2**
- 10 fishes were sampled and held out of water for 30 s and then transferred to a new tank with a dramatic level of water by 0.5 h

**Step 3**
- 10 fishes were sampled and held out of water for 30 s and then transferred to a new tank with a dramatic level of water by 3.0 h

**Step 4**
- Similar to Step 3, but with the modification that after fishes were returned to normal conditions and sampled after 24 h

**Tissues:**
- Plasma: Cortisol Analysis
- Kidney, Spleen, Liver and Muscle: Fluorescence - MSAP analysis.
## MATERIALS AND METHODS

**Amplified DNA fragments**

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Represents the band presence for both enzyme combinations</td>
</tr>
<tr>
<td>UNMETHYLATED</td>
<td></td>
</tr>
<tr>
<td>Type II</td>
<td>Band presence only for EcoRI/HpaII</td>
</tr>
<tr>
<td>HEMIMETHYLATION</td>
<td></td>
</tr>
<tr>
<td>Type III</td>
<td>Band presence for EcoRI/MspI</td>
</tr>
<tr>
<td>FULL METHYLATION</td>
<td></td>
</tr>
<tr>
<td>Type IV</td>
<td>Represents the band absence for both enzyme combinations</td>
</tr>
</tbody>
</table>

### Statistical Analysis

- **AMOVA**
- **PCoA (Principal Component Analysis)**
MATERIALS AND METHODS

Draft assembly of the Atlantic salmon genome (NCBI Assembly) → IN SILICO EVALUATION → Non-redundant unigenes from public databases

IN SILICO EVALUATION

CpG<sub>O/E</sub> RATIO (Takai and Jones, 2002)

NOCOM program to test whether the gene frequency distribution differed significantly from a unimodal distribution

Invertebrate model

Vertebrate model

Draft assembly of the Atlantic salmon genome (NCBI Assembly)

Non-redundant unigenes from public databases

Robinson et al., 2011

Okmura et al., 2011
IN SILICO EVALUATION OF CpG RATIOS
ATLANTIC SALMON GENOME

- The genomic distribution of CpGo/e ratio were clearly unimodal
- One peak focused at approximately in 0.25.
- This suggest that Atlantic salmon genome present a global pattern of methylation, characteristic of vertebrates (Where a great percentage of CpG sites are methylated and a reduced CpG sites, probably localized in CpG islands, are unmethylated.)
RESULTS

Patterns and levels of DNA methylation in four tissues of Atlantic STRAINS/TISSUE POOLED

- Full methylation ratio
- Hemimethylation ratio
- Unmethylation ratio

Type III (full methylation) pattern most frequent (AMOVA Φ ST = 0.0432, p<0.01),
IN SILICO METHYLATION ANALYSIS – EST AVAILABLE ATLANTIC SALMON

- LEUKOCYTE MIGRATION
- LEUKOCYTE ACTIVATION
- T CELL SELECTION
- LYMPHOCYTE COSTIMULATION
- ANTIGEN PROCESSING AND PRESENTATION
- IMMUNE RESPONSE
- IMMUNE SYSTEM DEVELOPMENT
- TOLERANCE INDUCTION
- B CELL SELECTION
- ACTIVATION OF IMMUNE RESPONSE
- IMMUNE EFFECCTOR PROCESS
- SOMATIC DIVERSIFICATION OF IMMUNE RECEPTORS
- LEUKOCYTE HOMEOSTASIS
- IMMUNE SYSTEM PROCESS
- KILLING OF CELLS OF ANOTHER ORGANISM
- LEUKOCYTE MEDIATED CYTOTOXICITY

CpG Ratio
CORTISOL LEVELS

*: p<0.05
RESULTS

GLOBAL DNA METHYLATION WITHIN AND AMONG TISSUES
STRAINS POOLED

No significant among tissues (AMOVA p<0.01; PCoA),
RESULTS

DNA METHYLATION AMONG STRAINS

Only SPLEEN showed differences among strains (AMOVA $\Phi_{ST}=0.0832$, $p<0.01$), PCoA confirm.
RESULTS

DNA METHYLATION WITHIN STRAINS

GASPE STRAIN

LAKS STRAIN

Only THE PAIR KIDNEY/SPLEEN NO showed differences WITHIN strain.
RESULTS: DIVERSITY AND PROFILES EXPRESSION BY TISSUE (STRAINS POOLED) *; p<0.05 - control

KIDNEY

LIVER

MUSCLE

*; p<0.05 - control
ATLANTIC SALMON SHOW A GREAT DIVERSITY OF METHYLATION CHANGES THAT AGREE WITH THE DIVERSITY SHOWED IN RAINBOW TROUT (Blouin et al, 2009).

FULL METHYLATION IS THE STATE MORE FREQUENTLY

SPLEEN WILL BE A IMPORTANT ORGAN TO EVALUATE DIFFERENT PATTERN OF METHYLATION AMONG STRAIN

STRESS SHOW A CLEAR EFFECT IN THE PATTERNS OF METHYLATION

STUDIES OF GENE EXPRESSION SHOULD INCORPORATED ANALYSIS OF METHYLATION

EPIGENOMICS WILL BE A IMPORTANT ASPECT IN ANIMAL BREEDING BECAUSE IT MAY HELP IDENTIFYING PART OF THE MISSING CAUSALITY AND MISSING HERITABILITY OF COMPLEX TRAITS AND DISEASES
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