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IMPACT OF TEMPERATURE REGIME DURING EMBRYONIC DEVELOPMENT ON GENOMEWIDE PATTERNS OF DNA METHYLATION IN LIVER SAMPLES OF JUVENILE RAINBOW TROUT

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Introduction

Epigenetic mechanisms are involved in the long-term persistence of physiological effects resulting from events that occurred earlier in the life of an animal. We aim to investigate the potential role of epigenetic marks in the expression of phenotypes and their variability in fish, in particular to study whether the epigenetic marks established in response to an environmental stress depend on the genetic background. In this context, rainbow trout isogenic lines (Quillet et al. 2007) are the material of choice. Within each line, all fish have the same genome i.e. there is no genetic variability. This allows the comparison of epigenetic marks among several individuals with the same genotype. The environmental stress chosen here is temperature, a known induction factor of epigenetic marks in fish. A recent study has shown that temperature experienced during development has prolonged effects on DNA methylation levels throughout the genome of threespine stickleback (Metzger and Schulte 2017). We have demonstrated that thermal history during embryonic development alters genome-wide patterns of DNA methylation at eyed-stage, but to a greater or lesser extent depending on the genetic background (Lallias et al. 2020). The objective of this study was to investigate the persistence in time of genome-wide patterns of DNA methylation established in response to early temperature regime in rainbow trout isogenic lines.

Material and methods

Eight rainbow trout isogenic lines were produced at INRAE PEIMA. For each line, half of the eggs were incubated at standard temperature (11°C) and the other half at high temperature (16°C), from eyed-stage to hatching. Just before hatching and for the rest of the rearing, all batches were reared at 11°C. Liver samples (central organ for intermediary metabolism) were collected on 4 month-old juvenile fish, snap frozen in liquid nitrogen and kept at -80°C until DNA extraction.

Global methylation levels were quantified using LUminometric Methylation Assay (LUMA) for 80 juvenile fish (8 lines x 2 temperature regimes x 5 fish per condition). Statistical analyses were performed using non-parametric tests suited for small samples (permutation tests for two/K independent samples with Monte-Carlo sampling; coin plug-in in RCommander).

Genomewide patterns of DNA methylation were analysed by Reduced Representation Bisulfite Sequencing (RRBS) on 40 juvenile fish (4 lines x 2 temperature regimes x 5 fish per condition). RRBS libraries were prepared on the same DNA extracts used for LUMA and then sequenced on an Illumina NovaSeq6000 sequencer to produce 100 bp paired-end reads (Integragen SA, France). Trimmed reads were aligned to the current reference genome with the bisulfite mapping tool Bismark. Differential methylation analyses were performed using methylKit. Identified DMCs (Differentially Methylated Cytosines) and DMRs (Differentially Methylated Regions) were finally annotated. All

steps are monitored using a homemade pipeline previously described (Perrier et al. 2018).

Results

As for results obtained at eyed-stage, there was no overall effect of temperature regime (11°C vs 16°C) experienced during embryonic development on global DNA methylation of liver samples of 4 month-old juvenile fish ($z = 1.5705$; $p=0.116$) but significant differences between lines at 11°C (chi-squared = 15.224; $p=0.033$) and 16°C (chi-squared = 18.586; $p=0.010$).

An average per individual of 41 million paired-end reads were obtained (lowest: 23 million; highest: 77 million). Bisulfite conversion rates were very high (>99%). Total mapping efficiency of the RRBS reads to the reference genome ranged between 74 and 79%, but only 50% on average mapped uniquely and were used for subsequent analysis. Preliminary results reveal that the numbers of DMCs and DMRs are relatively low but vary greatly depending on the line. Further analysis is ongoing.

Discussion

Rainbow trout isogenic lines are a unique and powerful biological model to study whether the methylation marks established in response to an environmental stimulus (temperature here) depend on the genetic background, persist in time (several months after exposure) and can impact the response to later challenges. Our results suggest some level of persistence in time of methylation marks established in response to an early temperature stress.

Acknowledgements

This study was carried out within AQUAEXCEL^{2020} funded by European Union's Horizon 2020 research and innovation programme under grant agreement No 652831. We are grateful to the Genotoul bioinformatics platform Toulouse Occitanie (Bioinfo Genotoul, doi: 10.15454/1.5572369328961167E12) for providing computing and storage resources.

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