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Characteristics of *fads2* gene expression and putative promoter in European sea bass (*Dicentrarchus labrax*): Comparison with salmonid species and analysis of CpG methylation

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ABSTRACT

Marine fish species exhibit low capacity to biosynthesise highly unsaturated fatty acid (HUFA) in comparison to strict freshwater and anadromous species. It is admitted that the Delta(6) desaturase (FADS2) is a key enzyme in the HUFA biosynthetic pathway. We investigated by quantitative PCR the relative amounts of FADS2 mRNA in European sea bass (*Dicentrarchus labrax*) in comparison with a salmonid species, the rainbow trout (*Oncorhynchus mykiss* L.). The analysis of the expression data was performed regarding the difference of the characteristics of a critical fragment of the *fads2* gene promoter between sea bass and Atlantic salmon. The lower level of *fads2* gene expression observed in sea bass suggested that *fads2* gene putative promoter, which exhibited an E-box like Sterol Regulatory Element (SRE) site but lacked a Sp1 site, is less active in this marine species. The cytosine methylation of CpG sites in the putative promoter region including E-box like SRE and NF-Y binding sites of sea bass *fads2* gene was also investigated following a nutritional conditioning of larvae. However, no significant difference of CpG methylation could be found for any of the 28 CpGs analysed between larvae fed diet with high or low HUFA contents. In conclusion, the present data revealed lower constitutive expression of the *fads2* gene possibly related to different characteristics of gene promoter in sea bass in comparison with salmonid species, and indicated that long-term conditioning of *fads2* gene expression did not influence the methylation of the gene promoter at potential SRE binding site.

1. Introduction

It is well documented that strict marine fish species exhibit lower HUFA biosynthesis capacities than anadromous species such as salmonids (Leaver et al., 2008). This characteristic is highlighted in experiments investigating the impact of dietary fish oil substitution by vegetable oil on the flesh fatty acid contents and on some physiological parameters in different fish species (Menoyo et al., 2005; Montero et al., 2010; Montero et al., 2005; Peng et al., 2008). Marine fish are unable to convert PUFA into HUFA at a physiologically significant rate due to apparent deficiencies in one or more steps in the biosynthetic pathway (Ghioni et al., 1999; Tocher and Ghioni, 1999). The low level of HUFA biosynthesis from vegetable precursors in marine fish species including Atlantic cod (*Gadus morhua* L.) and European sea bass (*Dicentrarchus labrax* L.) could be explained by a deficiency in FADS2 activity (Geay et al., 2010; Tocher et al., 2006). Since *fads2* gene has been cloned from several marine fish species (Zheng et al., 2004), the regulation of its expression has been examined during the last decade.

Globally in eukaryotes, the regulation of gene expression can occur at different steps ranging from DNA–RNA transcription to post-translational modification of protein (O'Malley et al., 1977). Transcription is promoted by a DNA sequence, called promoter, typically located upstream from the start site of transcription. The level of gene transcription is greatly dependant on the binding of RNA polymerase and transcription machinery on specific sequences of the promoter. The regulation of gene transcription can thus involve the interaction of transcription factors with the transcription machinery as well as changes in DNA structure (epigenetic process including CpG dinucleotide methylation) which influence accessibility of promoter sequences. It is now well admitted that some transcription factors such as Sterol Regulatory Element Binding Proteins (SREBPs) can act as nutrient sensor to stimulate the basal *fads2* gene transcription in vertebrates (Nakamura and Nara, 2002). It has also been shown in mammals that a regulation of the methylation in a CpG rich region of the *fads2* gene promoter can modulate its expression contributing to the pathology of hyperhomocysteinemia (Devlin et al., 2007).

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In the cod, the deficiency in FADS2 activity can be explained by both a decrease of the level of basal expression and nutritional regulation of *fads2* gene compared to Atlantic salmon (Tocher et al. 2006). The comparative analysis of *fads2* gene promoters of salmon and cod performed by Zheng et al. (2009) indicated that this deficiency could be related to a less active promoter in cod. In this regard, the same authors identified a potential Sp1 transcription factor binding site required for basal *fads2* gene expression of the salmon which was absent in cod promoter. Sp1 has been shown in mammals to cooperate extensively with NF-Y and SREBP1 in order to coordinate the expression of thousands of genes involved in several metabolic pathways in liver (Reed et al. 2008). Contrary to cod, sea bass exhibits higher FADS2 and SREBP1 messenger amounts when fed vegetable oil (Geay et al., 2010; Gonzáles-Rovira et al., 2009). However, despite this stimulation of *fads2* gene transcription, the activity of the corresponding enzyme in sea bass liver is not enhanced (Mourente and Dick, 2002; Mourente et al., 2005) and is lower than the activities measured in salmonids (Zheng et al., 2005). A post transcriptional regulation of *fads2* gene expression in sea bass has been recently suggested to explain this discrepancy (Geay et al., 2010), but until now, little is known about the relative basal amounts of FADS2 transcripts in sea bass compared to salmonids.

It has been suggested that lower HUFA biosynthesis capacities could be explained in marine fish species by the availability of preys enriched in HUFA in their natural environment resulting in the absence of pressure and in dysfunction of the HUFA synthesis pathway throughout evolution (Tocher, 2003). Interestingly, Vagner et al. (2007) indicated that the *fads2* gene transcription could be positively and durably regulated by a nutritional conditioning during the larval stage in sea bass. The molecular mechanism involved in this induction is not known but could possibly involve gene imprinting through epigenetic regulation such as methylation of CpG dinucleotides.

In the present study, we have analysed by quantitative PCR the relative quantities of FADS2 messengers in the liver and intestine of sea bass compared with a salmonid species, the rainbow trout. In parallel, we have investigated the characteristics of a putative FADS2 promoter, the potential transcription factor sites and CpG rich islands from sequence recently obtained by Santigosa et al. (2010). Moreover, we hypothesised that cytosine methylation at CpG sites in the promoter region of *fads2* gene could be responsible for the nutritional conditioning of HUFA biosynthesis pathway during sea bass larval development. To test this hypothesis, we have analysed the methylation of CpG rich regions, including NF-Y and E-box like SRE sites from individual genomic DNA of fish larvae fed fish oil or vegetable oil based diets.

2. Materials and methods

2.1. Sea bass and rainbow trout juvenile breeding

Sea bass and rainbow trout were fed with two commercial diet in order to cover their respective nutritional requirements such as HUFA [For sea bass: crude protein: 43% dry matter (DM), crude fat: 20% DM, EPA + DHA 7.5% lipid. For rainbow trout: crude protein: 40% DM, crude fat: 23% DM, EPA + DHA 11.5% lipid]. Rainbow trout (473 g ± 45 g) were obtained from the experimental station of Sizun (INRA institute, France) while sea bass (565 g ± 62 g) were bred in the Ifremer institute (Brest, France). Sampling was performed in winter after 10–12 h fasting for both species. The relative quantity of FADS2 messengers was individually measured in the liver and intestine of five fishes (males and females) from each species.

2.2. Larvae conditioning

Two iso-lipid and iso-protein diets were formulated for the nutritional conditioning experiment (Table 1). Lipids in the control diet (diet H) were brought by fish origin ingredients completed by a blend of lecithin (soybean and rapeseed). In the experimental diet (diet L), most of fish meal was replaced by defatted fish meal while fish oil was substituted by soybean oil. Both diets provided similar balance of saturates, monoenes and PUFA but different HUFA contents. The fatty acid composition of the two diets is given in Table 1. Sea bass larvae obtained from the Station of Palavas-les-Flots (Ifremer institute, France) were distributed randomly into five 35 L cylindrical tanks at 2800 fishes per tank. The tanks were supplied with non-recirculated seawater at a constant temperature of 20 °C and were subjected to a photoperiod of 12 h light:12 h dark. Both diets were distributed ad libitum automatically 12 h/day during all the larvae stage. After the metamorphose (around 45 days post-hatching), all fish nourished with the same diet were pooled in a 1000 L tank and after an acclimatization period of 2 days, 20 fishes per condition were sampled for liver analysis. Real time PCR investigation of FADS2 and 18S in liver was performed from 20 samples per group while methylation analysis of the FADS2 promoter was investigated with 10 samples per group.

Table 1
Ingredients (% dry matter) and fatty acids composition (% sum of fatty acids) of the diets containing high (H) and low (L) HUFA levels.

Diets	H	L
Ingredients (% DM)		
Fish meal	54.0	16.5
Defatted fish meal	0	36
CPSP 90	10.0	10.0
Soybean oil	0.0	3.5
Lecithin blend (soybean + rapeseed)	21.0	23.0
Marin phospholipids	2.0	0.0
Vitamins premix	1.0	1.0
Minerals premix	1.0	1.0
Cellulose	2.0	0.0
Liant	1.0	1.0
Fatty acids composition (% sum of fatty acids)		
Σ Saturates	4.3	3.2
Σ Monoenes	5.2	5.0
18:2n−6	4.1	5.5
20:2n−6	0.0	0.0
18:3n−6	0.0	0.0
20:4n−6	0.1	0.0
Σ n−6 PUFA	4.2	5.5
18:3n−3	0.6	0.8
18:4n−3	0.2	0.0
20:3n−3	0.0	0.0
20:4n−3	0.1	0.0
20:5n−3	0.5	0.1
22:5n−3	0.1	0.0
22:6n−3	0.7	0.2
Σ n−3 PUFA	2.2	1.1
EPA + DHA	1.2	0.3
Total lipid	15.9	14.8

These experiments have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.3. RNA extraction and real-time quantitative-PCR analysis

Total RNA originated from either larval (liver) and adult (liver and intestine) tissues was extracted using Trizol reagent (Invitrogen, USA), and quantified by measuring absorbance at 260 nm in a spectrophotometer (Nanodrop Labtech, France). The reverse transcription was performed using the QuantiTect® Reverse Transcription kit (QIAGEN) including a genomic DNA elimination reaction. Reactions were carried out in 20 µl of volume containing 1 µg of total RNA, 1 µl Quantiscript Reverse Transcriptase, 4 µl Quantiscript RT buffer (5×), 1 µl Primer Mix and sterile MilliQ water. *Fads2* gene as well as the