Short scientific report

Abstract

Cryopreservation of spermatogonia stem cells (SSCs) are a great alternative for the preservation of fish genome. In zebrafish, these cells retain the migratory capacity after cryopreservation and culture, and therefore can colonize the genital ridge or gonad primordium once transplanted in the recipient embryo/larvae. These tools will be highly valuable both for fish farming and aquaculture research, allowing us to transfer some of these techniques to other problematic species such as the large pelagics, for example; meagre. Seed production from large fish with long generation times is expensive, because it requires extensive rearing space and is labour-intensive. If undifferentiated germ cells of such target species could be transplanted into closely related fish, which are smaller and have shorter generation times, such surrogate parent fish could support the proliferation of donor stem cells and the production of mature eggs and sperm from donor species. These attempts have been made by Dr Elsa Cabrita group and others and demonstrated to be a successful tool in the conservation of certain genomes and in the implementation of new strategies on fish reproduction of cultivated species. In this sense, Meagre seems to be an interesting specie for commercial purposes because it is a particularly lean fish, has a high dressing percentage, low adiposity, healthy muscular lipid content, and long shelf life. In addition, it reaches a relatively large commercial size quite rapidly.

Purpose of the STSM

For this reason in this STMS the main objective was to study the gonadal sex differentiation and first sexual maturation of Meagre, based upon the characterization and cloning of vasa molecular marker in this specie. Vasa is a specific molecular marker for spermatogonia and the sequence for turbot and Senegalese sole was annotated previously by Dr Elsa Cabrita group. This objective is a very valuable tool both for fish farming and aquaculture research.

Description of the work carried out during the STSM

Biological samples

Specimens of meagre (*Argyrosomus regius*) with different ages were caught in the IPMA (Olhao, Portugal) during the second spawing season (April to July, fish from 12 to 60 months). Fish for tissue collection were anesthetized using 2-phenoxyethanol (1500 parts per million). Dissection of the gonads were performed using fine forces.

Histological analysis

To evaluate developmental stage of the gonads, a histologic assay was perform. Samples from 12, 24 and 60 months were fixed in 4% paraformaldehyde (PFA). After fixation

the samples were washed three times during 20 min in phosphate-buffered saline (PBS). Samples were exposed to an increased ethanol concentration and stored at 4^{o}C in 75% ethanol. To obtain 5 µm serial sections, the samples were embedded in paraffin. Haematoxylin and eosin staining presence or absence of spermatogonia stem cells (SSCs) in juveniles and adults.

As expected and according to previous studies, the biggest percentage of SSCs population was found in 24 months juveniles, in this stage only spermatogonia are present and tubules are well developed. Early developing spermatogenesis activity is generalized in testes. At later stages, 60 months, the most of the cells corresponding to spermatozoa population, spermatogenic cysts at all stages of development are present SC, spermatids and spermatozoa can be observed. In 12 months juveniles, testes just after testicular differentiation. Testes lack a well defined tubular system.

Cloning of meagre vasa cDNA

For the preparation of total RNA, meagre gonads from different developmental stages (12,24 and 60 months) were frozen in liquid nitrogen and stored at -80 ^{o}C until their further use. Samples were processed with the Nucleo Spin RNA II Isolation Kit (Macherey Nagel, Germany) according to the manufacter´s protocol (including a DNase treatment). Total RNA concentration was assessed using a nanodrop (A260nm/A280nm ratio> 1.8) and integrity was assessed by electrophoresis in a 1% agarose gel.

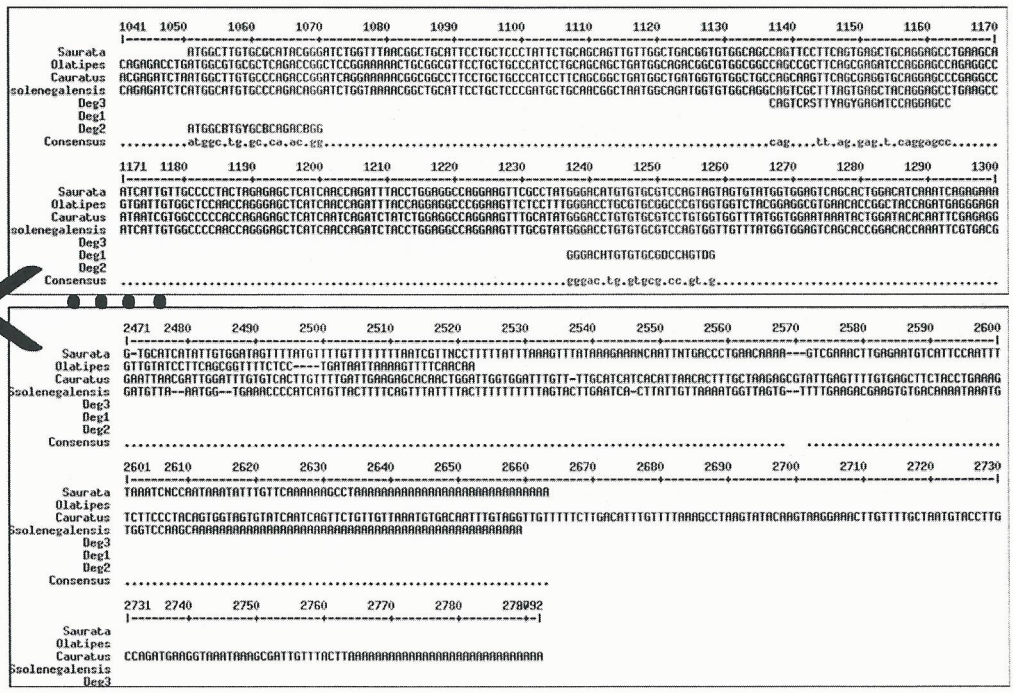
Meagre vasa molecular marker were isolated using total RNA from the gonads of 24 months juveniles using a commercial kits (Oligo Adapter and Advantage Polymerase Mix PCR kit, Clontech, EEUU). The first degenerated oligos were obtained from aligning the *O. mykiss* (AB032566.1), *O. niloticus* (AB032467.1), *O. latipes* (AB063484.1), *S. aurata* (AF520608.1), *D. rerio* (AB005147.1) and *C. auratus gibelio* (AY773078.1) vasa homologues from GenBank. Degenerated nucleotides: B = G;T or C; Y= C or T; H = A, C or T and D = A, G or T.

Vasa_teleosts-NFor GGGACHTGTGTGCGDCCHGTDG

Vasa_teleosts-For ATGGCBTGYGCBCAGACBGG

The third degenerated oligo was obtained from aligning the *O. mykiss* (AB032566.1), *O. niloticus* (AB032467.1), *O. latipes* (AB063484.1), *S. aurata* (AF520608.1), *D. rerio* (AB005147.1) and *C. auratus gibelio* (AY773078.1) and *Solea senegalensis* (JN564594) vasa homologues from GenBank. Degenerated nucleotides:B = G;T or C; Y= C or T; H = A, C or T and D = A, G or T.

In all cases, the PCR products were extracted from 1 % agarose gel using the Nucleo Spin Extract II kit (Macherey Nagel, Germany). Purified PCR products were cloned into pCRII vector (Invitrogen, Madrid) and sent for sequenciation.



Future collaboration with the host institution

A new longer STSMs will be request to characterize the vasa (previously sequenced in meagre) expression in different developmental stages using qPCR. Moreover a new vasa_in situ hybridization analysis in different developmental stages will be included in order to publish all results, including the acknowledgments to COST ACTION.

Confirmation by the host institution of the successful execution of the STSM

Dr Sonia Martínez Páramo, Post Doc of CCMAR, Aquaculture Research group in the University of Algarve, certifies that Dr Marta F Riesco visited us as a guest researcher from 1st April to 31st of May, 2014, thanks to a grant awarded by Short Them Scientific Mission, COST Action FA 1205.

(The Supervisor)

(The Applicant)