Final report for Short-Term Scientific Mission (COST Action: FA1205 – Aquagamete)
COST Reference Code: COST-STSM-ECOST-STSM-FA1205-170613-030343

Project data

Title: RNA isolation from different fish spermatozoa
Beneficiary: Ágnes Ősz, Szent István University - Department of Aquaculture
Host: Ferenc Müller, University of Birmingham - Department of Medical and Molecular Genetics
Period: from 17/06/2013 to 28/07/2013 Place: Birmingham (United Kingdom)

Purpose of project

1. To optimize fish sperm collection and preparation methods suitable for RNA isolation (trizol, liquid nitrogen etc.).
2. To learn an effective method for the isolation of RNA from small amounts of fish sperm.
3. To learn a method which allows testing the quality of isolated RNA. Assessment of isolated RNA for suitability for further use (for example production of cDNA, for sequencing and functional studies).

Work during the STSM period

1. Collection and preparation method: We have collected sperm from five common carp *(Cyprinus carpio)* individuals *using two methods* (with catheter and without catheter) one day after hormone injection. We have kept the sperm on ice during the preparation method. After collection we have divided the sperm into cryotubes in various amounts depending on the preparation method (50 µl sperm with 500 µl trizol, 100 µl sperm with 1000 µl trizol; 150 µl, 300 µl, 500 µl, 1000 µl undiluted sperm only in dry ice.) The undiluted samples have been centrifuged after dividing then the supernatant has been removed. All tubes have been frozen in dry ice then they were kept at –80°C. We have counted the cell amount in Burker chamber.
2. RNA isolation method: We have tried different RNA isolation method for comparison. We have isolated RNA from samples frozen with trizol and from undiluted samples. We have put 10 times trizol to the undiluted samples after thawing. RNA isolation from different samples was performed with homogenization as well as without homogenization in trizol. After these we have used the same RNA isolation method in all different samples (general RNA isolation protocol with trizol – web1). We have used 10 hpf embryos to control (high volume of somatic RNA). The results have been collected in a table.
3. Testing the quality of the isolated RNA: We have made cDNA with reverse-transcriptase PCR for testing the RNA isolation quality. We have tried to find some genes in carp that are specific in sperm and others are definitely not in sperm based on scientific articles. We have found the MSAP that is a sperm-specific gene in common carp (Ju, 2004), and beta-globin that is not in sperm (Lu, 2011). We have designed primers on the junction of the exons of these genes, so we can avoid the wrong transcription if we have DNA contamination.

Results

1. Collecting and preparation method: We have collected large volume of sperm after hormone injection. The samples have looked clear virtually, however the quality testing have not proved this (see below at step 3). This step need further improving.
2. RNA isolation method: We have isolated different volumes of RNA from the different samples. We have counted effectivity of RNA isolation based on data (see Figure 1.). We have found that the isolations with homogenization were much more effective than the isolations without homogenization. This is a very important step in the protocol. We have not been able to find any difference between the effectivity of isolation from sperm frozen with trizol and sperm frozen without solution.
3. Testing the quality of the isolated RNA: The primers have worked really well, however we have got RT-PCR product in all primers (sperm-specific as well as definitely not in sperm genes). We have assumed that maybe there have been some blood contamination in the sperm, or maybe beta-globin there are in the sperm in carp. We need to find other genes that are definitely not in sperm (for example keratin), but unfortunately the carp genome is not accessible yet.

Future plan

We keep in touch certainly with the host institution. We would like to improve the collection and quality testing methods, and try to isolate RNA from zebrafish sperm. Finally we would like to sequence the sperm RNA from common carp and zebrafish. If is it possible, I would like to visit the host laboratory again next year.

References

web1: http://tools.invitrogen.com/content/sfs/manuals/trizol_reagent.pdf

Ju, T., Huang, F. (2004): MSAP, the Meichroacidin Homolog of Carp *(Cyprinus carpio)*, Differs from the Rodent Counterpart in Germline Expression and Involves Flagellar Differentiation, Biology Of Reproduction 71, 1419–1429 (2004)

Lu,W., Mayolle, A., Cui, G., Luo, L., Balment, R. J. (2011): Molecular Characterization and Expression of α-Globin and β-Globin Genes in the Euryhaline Flounder *(Platichthys flesus)*, Hindawi Publishing Corporation, Evidence-Based Complementary and Alternative Medicine, Volume 2011, Article ID 965153, 11 pages

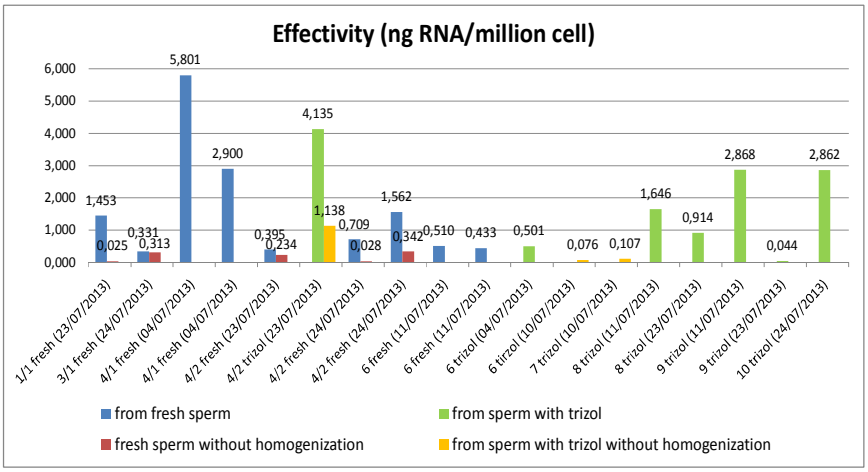

Figure 1.: The effectivity of RNA isolation from common carp sperm with different samples and methods