

## **Proteome analysis of Atlantic cod larvae (*Gadus morhua*) treated with peptides and probiotic bacteria.**

Hólmfríður Sveinsdóttir and Ágústa Guðmundsdóttir, Science Institute, University of Iceland. holmfrs@hi.is

**Introduction:** The aim of the project is to use proteomics to create new knowledge on the effects of peptides and probiotic bacteria on the expression of trypsin as well as other proteins in Atlantic cod larvae (*Gadus morhua*). Studies on marine fish larvae have shown that the first days of larval feeding are characterized by slow growth and high mortality. One of the main reasons is poor digestive function and trypsins are known to play a key role in the digestive function of early stage larvae. In this study, an attempt was made to use peptides and probiotic bacteria to improve the digestive function of cod larvae as these treatments may increase trypsin expression and activity.

**Materials and methods:** Rearing experiments were undertaken at the Marine Research Institute, Staður, Grindavík. Newly hatched larvae were divided into three groups: C-group (Control), P-group and B-group. Each group contained 7500 larvae per 150 l tank. P-group: 5 g of fish peptides were added daily to the seawater for the first 8 days post hatch (ph), B-group: 6 g of probiotic bacteria mixture (REMUS from Avecom, Belgium) were added every other day to the seawater throughout the experiment (24 ph). B-group was also fed once a day with rotifers (*Brachinous plicatilis*) pre-inoculated with the probiotic bacteria mixture at a concentration of 12 g/l 1 rotifers. The larvae were maintained in aerated seawater at 9°C, flow rate of 750 ml/min and continuous light (300 lux on the water surface) throughout the experiment. Larvae were fed 3 times a day with rotifers (1 l rotifers/tank) and twice a day with algae (2.5 ml/tank). The survival rate and dry weight of the larvae were estimated at the end of the experiment (24 ph). Samples for proteome analysis were collected on day 6 ph from the P-group and on day 24 ph from the B-group. Larvae samples were homogenized in four volumes of lysis buffer (7M urea, 2M thiourea, 4% (w/v) CHAPS, 0.3% (w/v) DTT, 1% protease inhibitor cocktail), followed by centrifugation. Soluble larvae proteins were mixed with re-swelling buffer (7M urea, 2M thiourea, 4% (w/v) CHAPS, 0.3% (w/v) DTT) and then added to a immobilised pH Gradient strip (IPG). Isoelectric focussing was performed in 3 stages with a ramped voltage change. For the second dimension electrophoresis, the IPG strip was laid onto a 12% gel (16×15 cm) and electrophoresed. The resolved proteins were detected using Collodial Coomassie Blue G250 staining. A control gel was made from isolated cod trypsin. The gels were scanned and subsequent analysis of the gel images was performed using the software package Phoretix 2D. The spots on the control gel were excised from the stained gel, digested with trypsin and the resulting peptide mixture was analyzed by MALDI-TOF mass spectrometry.

**Results and discussion:** The survival rate of larvae was 23.9% in B-group, 12.5% in P-group and 2.2% in C-group. The mean dry weight was  $0.74 \pm 0.06$  mg in B-group,  $0.62 \pm 0.04$  mg in P-group and  $0.82 \pm 0.09$  mg in C-group. Peptide mass fingerprint revealed two types of trypsins, cod trypsinogen I (MW 25811 Da, pI 6.2) and trypsinogen X (MW 25845 Da, pI 5.5) on the control gel. Spots, which were identified as trypsins, were present on the gels from all three groups. Further analysis, using the Phoretix 2D software, will reveal the efficacy of pretreatment with peptides and probiotic bacteria on the digestive function of Atlantic cod larvae.