

Egg quality in domesticated and wild seabass (*Dicentrarchus labrax*): A proteomic analysis

by

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ABSTRACT. - In order to improve our understanding of factors determining egg quality in fish, seabass egg batches obtained from 34 females held under standard conditions, were assessed for biological performances and a fraction from each batch was subjected to proteomic analysis. Comparison of proteomic patterns from eggs of different quality revealed differential protein spots among which one was identified as Nucleoside Diphosphate Kinase, a highly conserved protein involved in cell proliferation. This study shows that molecular markers may help understand factors determining egg quality in fish.

Key words. - Proteome - Egg quality - NDK - Seabass.

Introduction

Fish egg quality is highly variable and can be affected by several environmental factors and husbandry practices (Brooks *et al.*, 1997). The improvement and stabilization of quality require a better understanding of the molecular basis of egg developmental competence such as survival at various stages of embryonic and larval development, that remains poorly understood. Egg components such as maternally-inherited proteins support early development and their abundance in the eggs may be affected by environmental factors, husbandry practices and possibly, domestication. Egg proteome has been studied in zebrafish (Knoll-Gellida *et al.*, 2006) but never analyzed in relationship with the developmental competence of the egg.

Methods

Ovulations of wild and domesticated F3 females were stimulated with DTRP6-LHRHa at similar stage. Eggs were collected by stripping and fertilized *in vitro*. Fertilization rate and daily embryonic survival rates were subsequently recorded in order to fully characterize egg quality for each individual female. For each spawn, egg cytoplasmic proteins were delipidated by a two-phase extraction medium (Butanol/Diisopropylether; 4/6). Samples were then subject to IEF on 3-10 linear PH gradient 17 cm strip. The second migration was run through 12% SDS polyacrylamide gels. 2D protein expression patterns were analyzed using Image Master2D Platinum after Coomassie blue coloration. Differential spots were then analyzed by MALDI TOF/TOF mass spectrometry. Corresponding protein sequences were identified from MSDB database by Mascot software (Matrix science). An antibody directed against a portion of the sequence of a candidate protein, was raised in rabbits.

Results

Eggs originating from wild caught females showed higher fertilization rates (62.8%) than those of domestic females (41.4%, $p < 0.001$). Similarly, a higher hatching rate was observed in eggs from wild females (32.3% *vs* 16.1%, $p < 0.001$). Among the 100 protein spots that were detected on 2D gels, several domestication-linked or quality-linked spots were observed. Among them, one spot was identified by MS/MS as Nucleoside Diphosphate Kinase. Western blot analysis of seabass eggs revealed a single band at the expected size of 18 kDa. The abundance of this protein in eggs is positively linked to development potential.

Conclusion

The use of large-scale analytical methodologies such as proteomics to decipher the molecular basis of egg developmental competence is promising. The identification of specific proteins exhibiting a differential abundance in eggs of varying quality will contribute to the identification of the mechanisms determining egg quality defects. Moreover the markers can rapidly be used as objective molecular probes to assess the improvements of broodstock management.

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