Can *Artemia* produce essential fatty acids?

Roles of elongases in the biosynthesis of long-chain polyunsaturated fatty acids

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INTRODUCTION

- *Artemia* nauplii are one of the most used live preys in marine larviculture
- However, suboptimal levels of essential fatty acids: EPA (20:5n-3), ARA (20:4n-6) & DHA (22:6n-3) are found.
- Thus, *Artemia* requires enrichment for increasing its nutritional value
- Nevertheless, LC-PUFA biosynthetic capacity remains unknown in *Artemia*

- Elongases (Elo) are key enzymes in the LC-PUFA biosynthetic pathways
- Elo enzymes enable the extension of 2-carbon atoms over the fatty acid substrate:

18:3n-3	20:3n-3	18:2n-6	20:2n-6
18:4n-3	20:4n-3	18:3n-6	20:3n-6
20:5n-3	22:5n-3	20:4n-6	22:4n-6
22:5n-3	24:5n-3	22:4n-6	24:4n-6

OBJECTIVE

Clarify the repertoire and function of elongases with putative roles in the LC-PUFA biosynthesis of *Artemia*

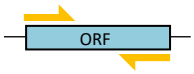
METHODS

Identification and molecular cloning of Elo sequences

Retrieving sequences

Data bases

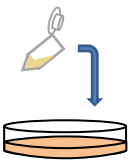
Amplification by PCR for molecular cloning



Insertion into expression vector

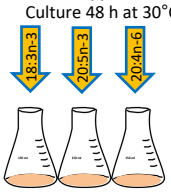
Transformation and functional characterisation

Yeast transformation. Culture 72 h at 30°C



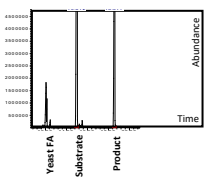
Pre-culture in LB broth. Culture 72 h at 30°C

Substrate supplementation. Culture 48 h at 30°C



Fatty acid analysis

Analysis of yeast fatty acids by GC-MS



% of conversion

$$\frac{Product\ area}{Substrate + Product\ area} \times 100$$

RESULTS

Molecular analysis

- Six elongases were identified (Elo1-6)
- Elo1 can be classified in the group of Elovl4, an Elo type that is widespread in animals
- Elo2-6 belong to a group of unclassified elongases arbitrarily named "novel" elongases, which have been found extensively in crustaceans
- *A. franciscana* Elo genes share the motif [(Q/H)(V/I/L)(S/T)(F/L/V)LH(V/I/L)(Y/V/I)HH], which is characteristic from PUFA elongases

Elo1	QLSFLHIYHH
Elo2	QVSVLHVIHH
Elo3	HVSVLHVIHH
Elo4	HVSVLHVIHH
Elo5	QITLLHLVHH
Elo6	QISVLHLVHH

Functional characterisation

Table 1. % of conversion of supplemented PUFA substrates into elongation products.

Substrate	Product	Elo1	Elo2	Elo3	Elo4	Elo5	Elo6
18:3n-3	20:3n-3	0.75	0.57	nd	nd	0.45	0.45
18:2n-6	20:2n-6	0.29	0.39	nd	0.59	0.51	0.84
18:4n-3	20:4n-3	1.09	0.92	0.36	nd	0.58	2.47
18:3n-6	20:3n-6	0.33	0.52	1.66	0.30	0.43	1.55
20:5n-3	22:5n-3	0.12	0.12	nd	nd	0.03	0.24
20:4n-6	22:4n-6	nd	0.11	nd	nd	0.06	nd
22:5n-3	24:5n-3	0.12	0.66	nd	nd	0.07	nd
22:4n-6	24:4n-6	nd	0.25	nd	nd	0.30	nd

nd, not detected

CONCLUSIONS

- *Artemia franciscana* has at least six elongases (Elo1-6) that enable the elongation of PUFA substrates
- Functions of the *A. franciscana* Elo1-6 fulfill all the elongation steps required in the LC-PUFA biosynthetic pathways
- *Artemia* has some capacity for biosynthesis of essential fatty acids