Establishment of a fatty acid desaturase fads2 knockout marine fish strain and its effects

Naoki Kabeya*†[1], Chihiro Ishizaki[1], Kazunori Kimura[1], Shogo Sato[1], and Yoshiyuki Matsushita[1]

[1]Tokyo University of Marine Science and Technology – Japon

Résumé

Long-chain (> C20) polyunsaturated fatty acids (LC-PUFA), such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), are biosynthesised from α-linolenic acid (ALA, 18:3n-3) through sequential enzymatic reactions. Most marine Acanthopterygii fish possess only a limited set of enzymes within this pathway, which catalyse intermediate fatty acid synthesis but not the direct formation of EPA and DHA. Hence, the physiological significance of these enzymes remains unclear. The present study aimed to establish a *fads2* knockout (KO) strain in a marine Acanthopterygii fish Nibe croaker *Nibea mitsukurii* and analyse its effects. Using CRISPR/Cas9 with a microinjection technique, we generated F0 individuals, followed by F1 (*fads2+/+* : *fads2+/-* = 1:1), F2 (*fads2+/+* : *fads2+/-*: *fads2-/-* = 1:2:1), and an F3 generation consisting entirely of *fads2-/-* individuals. The fatty acid composition of *fads2-/-*F2 individuals and F3 larvae was compared with that of *fads2+/+* individuals. Fatty acid analysis revealed that, in the testes of F2 *fads2-/-* individuals, the level of 20:4n-3, an elongation product of 18:4n-3 synthesised via Fads2, was significantly lower than that in *fads2+/+* individuals (t-test, $P < 0.05$) (Figure 1). Similarly, 18:4n-3 and 20:4n-3 levels were significantly lower in F3 *fads2-/-* larvae (t-test, $P < 0.05$) (Figure 1). Despite these differences, *fads2-/-* individuals exhibited normal growth and maturation under standard rearing and seed production conditions, suggesting that *fads2*is not crucially important for development in *N. mitsukurii*. These findings highlight the importance of loss-of-function studies in precisely characterising the physiological roles of LC-PUFA biosynthetic enzymes in teleosts.

Mots-Clés: knockout, desaturase, EPA, DHA, biosynthesis

*Intervenant

†Auteur correspondant: naoki.kabeya@kaiyodai.ac.jp