クロマグロの性分化制御機構に関する研究

[課程博士・論文博士共通]

博士学位論文内容要旨
Abstract of Dissertation

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論文題目 Title	Studies on sex differentiation in Pacific bluefin tuna *Thunnus orientalis* （クロマグロの性分化制御機構に関する研究）		

Pacific bluefin tuna (*Thunnus orientalis*; hereafter, PBT) is one of the most important species for aquaculture worldwide, particularly in Japan. Currently, several research institutes and companies in Japan have achieved closed-cycle production of this species, and have made hatchery-produced tuna seed available for practical tuna aquaculture. Nevertheless, most bluefin tuna aquaculture practices continue to rely on wild-caught juveniles for seed stocks owing to the low productivity of closed-cycle aquaculture. As fish are endangered because of wild stock overfishing, the fishing of this species has been controlled based on an assessment by the Western and Central Pacific Fisheries Commission (WCPFC). There are numerous issues associated with the use of wild-caught juveniles for aquaculture, including the unstable supply and the negative impacts on wild stock management. For the conservation of this species and sustainable development of the tuna farming industry, closed-cycle aquaculture should be promoted; however, this requires development of technology to improve its productivity.

Sex manipulation in fish to produce mono-sex stocks is an important area in aquaculture research. Sex manipulation is a prominent tool for achieving several broad goals in aquaculture, including preventing precocious maturation and uncontrolled reproduction, taking advantage of differences in growth rate and economic value of the sexes, reducing the impact of phenotypic sex on product quality, increasing stability of egg production, and protecting the intellectual property of genetically improved strains. Previous studies have reported that in wild stocks of tuna species, including the Atlantic bluefin tuna (*T. thynnus*), southern bluefin tuna (*T. maccoyii*), bigeye tuna (*T. obesus*), albacore (*T. alalunga*), and PBT, males tend to be larger than females. These findings suggest that in tuna species, males exhibit higher growth performance than females in aquaculture settings. If male-cultured PBT exhibit higher growth performance than females, the production of mono-sex male stocks appears to act as a prominent tool to significantly improve the productivity of closed-cycle PBT aquaculture by shortening production times and reducing production costs.

Sex steroid hormones, specifically estrogens and androgens, play a critical role in fish sex differentiation. Furthermore, environmental, genetic, and social factors strongly influence sex differentiation in fish. Sex manipulation in fish can be achieved using several methods, such as sex steroid hormone administration and temperature treatment. Sex manipulation techniques are generally developed based on the knowledge of sex differentiation in target fish, because fish exhibit divergent sex differentiation mechanisms. Despite their ecological and commercial importance, there is almost no data available on sex differentiation mechanisms in tuna species, including PBT. Therefore, to develop appropriate intervention and control strategies for sex in cultured PBT, sex differentiation mechanisms in this fish need to be characterized in detail.

To demonstrate the mechanisms underlying gonadal sex differentiation, at first, this study identified the timing and process of gonadal sex differentiation in PBT using histological and immunochemical analyses. Gonadal sex differentiation in PBT was first histologically characterized by the formation of ovarian cavity in females and an efferent duct in males at 57 days post hatching (dph). The gonads were then directly differentiated into ovaries or testes according to the genotypic sex until 83 dph. Immunochemical analysis showed no sexual dimorphic proliferation of germ cells during early sex differentiation (41−57 dph),

suggesting that germ cell proliferation may not affect gonadal sex differentiation in PBT.

Then, this study identified the molecular mechanism that regulates gonadal sex differentiation in PBT. Based on comparative and temporal transcriptomic analyses, it is proposed that active Aromatase-mediated estrogen biosynthesis, which includes positive regulation of aromatase expression by Foxl2, induces ovarian differentiation in PBT. In contrast, Dmrt1 and Gsdf mainly promote testicular differentiation in PBT. Furthermore, *sult1st6y* (sulfotransferase family 1, cytosolic sulfotransferase 6y), a male-specific homolog of estrogen sulfotransferase, is specifically expressed in male PBT gonads during testicular differentiation. Sulfotransferases deactivate endogenous estrogens by sulfating them. It is speculated that Sult1st6y would trigger testicular differentiation through estrogen deactivation in male gonads at the onset of gonadal sex differentiation, and estrogen deficiency induces the upregulation of the genes promoting testicular differentiation, particularly *dmrt1* and *gsdf*. Furthermore, androgen biosynthesis is upregulated in differentiating male gonads, suggesting that endogenous androgens may also be vital for testicular differentiation.

Finally, this study investigated sexual dimorphism in the growth performance of aquaculture-produced PBT. A comparison of the body size between the sexes revealed that male-cultured PBTs were larger than females at harvest, suggesting that mono-sex male production may be a prominent tool for improving the productivity of closed-cycle PBT aquaculture by shortening production times and reducing production costs. Furthermore, this study developed techniques for sex manipulation of PBT based on knowledge accumulated from histological and transcriptomic analyses. Histological analysis identified timing of gonadal sex differentiation in PBT. Results of transcriptomic analyses suggested that Aromatase plays an important role in ovarian differentiation in PBT. In cases where estrogen synthesis by Aromatase is essential for ovarian differentiation, the suppression of Aromatase activity would be effective in inducing sex reversal of genotypic females into phenotypic males in PBT. Based on these findings, this study orally administered aromatase-inhibitor (letrozole) into juvenile PBT during the labile period, when the gonads differentiate. As a result, this study demonstrated that sex can be controlled in PBT through the oral aromatase-inhibitor administration to sexually undifferentiated fish, which resulted in 100% sex reversal of genotypic females into phenotypic males.

In conclusion, this study for the first time shows the comprehensive mechanisms underlying gonadal sex differentiation, superior growth performance of males compared to that of females in aquaculture settings, and the development of an all-male production technique in PBT. This study offers important insights into the establishment of mono-sex male aquaculture in PBT. In addition, the discoveries made herein provide theoretical and empirical foundations for understanding sex differentiation mechanisms in tuna species.