（学位第３号様式）

学 位 論 文 要 旨

氏　名	Megumi Matsumoto
題　目	Development of the vaccine for inducing cell-mediated immunity by using interleukin-12 against intracellular bacterial infection of fish (Interleukin-12 を指標とした魚類の細胞内寄生細菌感染症に対する細胞性免疫誘導型ワクチンの開発)

Cultured yellowtails (yellowtail *Seriola quinqueradiata*, amberjack *S. dumerili*, and king fish *S. lalandi*) account for about 70% of all amount of production in Japan, and Kagoshima prefecture is producing 30% of all yellowtails production. On the other hand, five hundred million yen per year has been lost by fish disease, which is equivalent to 30% of all yellowtail production. Intracellular bacterial diseases, Nocardiosis and Mycobacteriosis, found in yellowtails cause the most serious damages to the cultured yellowtails. Control of chemotherapy or traditional vaccine for aquatic animals cannot show enough effect against these diseases. Therefore, the yellowtail cultures have been eager for prevention a novel vaccine. The induction of cell-mediated immunity (CMI), which directly kills infected cell, is important in protecting from these diseases even in fish. In this study focused interleukin-12 (IL-12), which is the important cytokine to induce CMI in mammals, and tried to develop a novel vaccine against Nocardiosis in amberjack using IL-12 as a marker of CMI induction.

Mammalian IL-12 protein (called IL-12p70) is composed of an alpha chain (p35a) and a beta chain (p40). In yellowtails, two *p35* (*p35a* and *p35b*) and three *p40* (*p40a*, *p40b* and *p40c*) was identified, and all combination of p35 and p40 isoforms (six types of recombinant IL-12p70; rIL-12p70) were related to CMI induction in amberjack. Thus, the cocktail of six types of rIL-12p70 was added to the conventional vaccine, and the effect as a CMI-inducible vaccine against Nocardiosis was examined. After challenge test, 88% amberjack survived whereas 0% survived in the conventional vaccine injected fish. However, application of these recombinant is difficult because of the cost and the technique. Therefore, the author tried to clarify the endogenous amberjack IL-12 production mechanism in order to produce IL-12 using other materials. The phenomenon that only formalin-killed cell (FKC) could not induce *IL-12* production was focused and then, important factor of IL-12 production was examined. As a result, the IL-12 production was controlled by 1) *IL-12p35a* gene up-regulation and 2) phagocytosis of antigen by neutrophils in amberjack. In addition, 3) cell condition inside of the cell, particularly cell-wall component or outer lipids layer, induced *IL-12p35a* expression. Then, the author compounded a conventional vaccine and cell-wall lipids, followed by challenge test. After challenge test, 54% of all amberjack survived, indicating that cell-wall lipids are available for novel CMI-inducible vaccine.

The vaccine for Nocardiosis should be prevented from not only initial infection but also later-stage infection by reactivation of bacteria. Therefore, the development of multi-stage subunit vaccine is important in the future.