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Marker-Assisted Breeding for Viral Disease Resistance in Japanese Flounder (*Paralichthys olivaceus*)

Takashi Sakamoto^{1*}, Kanako Fuji¹, Kazunobu Kobayashi¹, Osamu Hasegawa², Akiyuki Ozaki³, and Nobuaki Okamoto¹

¹ Department of Aquatic Biosciences, Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan

² Kanagawa Prefectural Fisheries Research Institute, Kanagawa 238-0237, Japan

³ National Research Institute of Aquaculture, Fisheries Research Agency, Mie 516-0193, Japan

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Extended Abstract

DNA marker technologies can be used for genetic improvement through selection of favorable traits such as disease resistance. These traits are generally modeled as being controlled by many genes of small additive effects, known as quantitative trait loci (QTL). Construction of a genetic linkage map based on DNA markers at a large number of sites in the fish genome is necessary to identify QTL controlling traits of disease resistance. By identifying markers associated with high performance QTL in different strains or species, it may be possible to improve the performance of such traits in other strains through introgression of the desired QTL. One of the goals of selective breeding programs is to integrate genetic marker information from pedigreed brood stock into successful management and culture. Such an approach, termed marker-assisted selection (MAS) and/or marker-assisted gene introgression (MAI), is expected to increase genetic response by affecting efficiency and accuracy of selection.

Japanese flounder (*Paralichthys olivaceus*) is an economically important food fish, widely cultured in Asian countries such as Japan,

Korea, and China. Lymphocystis disease (LD) is caused by LD virus (LDV; family Iridoviridae) and has become widely spread in these countries, seriously damaging fish farms. Japanese flounder affected with LD develop characteristically hypertrophied cells, called lymphocystis cells, on skin, fins, and/or the mouth (Fig. 1). Fish with lymphocystis cells lose commercial value because they are ugly. Lymphocystis cells on the mouth prevent proper feeding and, in the worst case, can lead to starvation. There is no effective treatment or commercially available vaccine for LD. To solve this problem, we initiated a genetic linkage study to search for markers associated with LD resistance. As a first step, we constructed a primary genetic linkage map in Japanese flounder with 111 microsatellite markers and 352 AFLP fragments. The parental male linkage map consists of 25 linkage groups while the female map consists of 27 groups, with an average resolution of 8 and 6.6 cM, respectively. We identified linkage among 96% of the markers and the total map length was estimated to be 1000-1200 cM (Coimbra et al., 2003). A second-generation genetic linkage map consisting of

* Corresponding author. Tel/fax: +81-3-546-30450; e-mail: takashis@s.kaiyodai.ac.jp

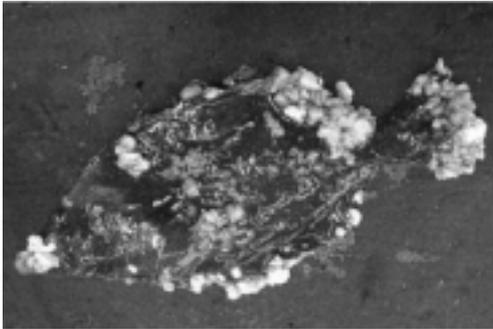


Fig. 1. Japanese flounder affected by lymphocystis disease, with tumor-like nodules of accumulated lymphocystis cells.

approximately 800 microsatellite markers has been constructed and more markers are currently being added to the map.

We tried to identify the LD-resistance locus (LD-R) by linkage analysis because it may facilitate the establishment of LD-resistance strains by MAS. We used 50 microsatellite markers to search for a locus associated with resistance to LD in Japanese flounder. Linkage analysis of LD resistance was conducted in a backcross progeny ($n = 136$) produced by crossing a susceptible male with a (susceptible \times resistant) hybrid female. Fish were reared with UV-treated water ($4 \times 10,000 \mu\text{W}/\text{cm}^2$, UV125, Hanovia) until the infectivity trials because ordinary rearing water is conta-

minated with LDV. The UV treatment completely protected the fish against LD. The LD-resistance test (phenotypic measurements) was carried out from February to May, 2001, during which time the backcross progeny were exposed to LDV-contaminated water. Fish were kept in a 5-ton tank with running water. Water temperature was 14-20°C during the experiment. The phenotypes were scored as either LD+ (fish that developed lymphocystis cells on skin, fins, and/or mouth) or LD- (fish without lymphocystis cells on skin, fins, or mouth). The backcross family was scored in May 2001.

One major locus (*Poli9-8TUF*) for LD resistance was detected on linkage group 15 of the Japanese flounder genetic linkage map (Fuji et al., 2006). When 136 fish were tested, the resistant allele (showing a 147 bp band for *Poli9-8TUF*) was inherited by 88.5% (54/61) of the healthy LD- progeny and by 17.3% (13/75) of the LD+ progeny (Fig. 2.). This locus explained 50% of the total phenotypic variation in the 136 screened individuals. Our results imply that LD resistance is inherited as a dominant trait that follows Mendelian inheritance. In the F_1 experiment, the number of LD+ and LD- fish were 51.1% and 48.9%, respectively. The mechanism underlying resistance is often explained by the presence or absence of certain molecules in the host that are critical for infection, recognition, or elimination of the pathogen.

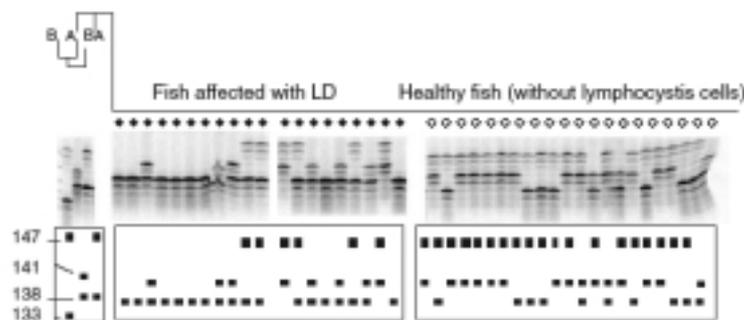


Fig. 2. Autoradiograph of one marker (*Poli9-8TUF*) associated with lymphocystis disease (LD) resistance on LG15. The upper band (147 bp) from B (a resistant strain) was confirmed to be responsible for LD resistance.

To introduce the trait and marker information linked to LD resistance into a commercial strain by MAI, we performed a cross between a resistant strain and commercial strain of Japanese flounder, and generated F₁ hybrid families. The LD resistant stock produced by MAI was tested on commercial fish farms. Until now, the results of the field tests on F₁ hybrid families demonstrated that LD resistance was successfully transmitted to the commercial strain. Our results show that MAI can be useful for genetic improvement through selection of favorable traits.

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