Molecular fingerprinting of olive flounder pathogenic *Streptococcus parauberis* strains by random amplified polymorphic DNA analysis

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Abstract

Two infectious species of Streptococcosis pathogens were detected by multiplex PCR assay. Detection rates of *Streptococcus iniae* and *S. parauberis* could reach 44.9% and 55.1% respectively for one year during 2004 to 2005 in Jeju island. These findings showed that *S. parauberis* strains were important pathogen with streptococcosis of olive flounder in Jeju island. These findings showed that *S. parauberis* strains were important pathogen with streptococcosis of olive flounder in Jeju island. In the present study we have investigated the interspecific relationship of all Jeju area of *S. parauberis* by RAPD analysis. Represent strains divided to four groups by RAPD fingerprints. The important differences observed between the olive flounder isolates suggest that they could constitute a well-differentiated group or a separate clonal line within this bacterial species. Though, serological research of *S. parauberis* strains in Jeju island not exist yet. These strains doing the serological evolution.

Introduction

Streptococcal diseases are serious problems for the fish culturing industry, in both fresh and marine waters, all over the world. Between 1993, the year of the first outbreak of streptococcosis in turbot (*Scophthalmus maximus*) by *Streptococcus parauberis*, and 1996, the disease caused important economic losses to the turbot industry in Spain.

We isolated and identified of the *S. parauberis* strains from cultured flounder in Jeju island. Besides Detection rates of *Streptococcus iniae* and *S.*

parauberis could reach 44.9% and 55.1% respectively for one year during 2004 to 2005 in Jeju island. However, The phenotypic, serological and phylogenetical characterization of flounder pathogenic *S. parauberis* strains in Jeju island has not yet existed.

Reliable methods for evolutional and serological strain differentiation are important for epidemiological studies. Discriminative methods based on genotypic differences are not affected by the physiological state of the organism and can be easily standardized.

Determination of random amplified polymorphic DNA (RAPD) patterns have been successfully employed for discriminating strains of a number of bacterial fish and shellfish pathogens and their potential use as epidemiological and typing tools has been demonstrated.

In this work, this technique have been evaluated in the investigation of strain heterogeneity within the population of *S. parauberis* isolated from different epizootic outbreaks in several olive flounder farms in Jeju island.

Bacterial strains and DNA extraction.

One hundred eighty seven *Streptococcus* sp. strains were used in this work. The represent strains and geographic origin of these strains are shown in fig. 1. Strains were routinely grown on brain heart infusion agar (Difco) plates at 25°C for 24 to 48 h.

DNA isolation was performed using *AccuPrep* Genomic DNA Extraction kit and allowed user manual (Bioneer, korea)

Identification of *S. parauberis* using by multiplex PCR assay.

The target gene and oligonucleotide primer set used for the detection of each of the three fish bacterial pathogens in the multiplex PCR are indicated in Table 1.

Multiplex PCR was carried out by mata *et al.*, (2004) method. The amplifications were carried out in a PCR gradient thermal cycler (TaKaRa) with the following parameters: an initial denaturation step of 94°C for 2 min; 25 serial cycles of a denaturation step of 92°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 90 s;

and a final extension step of 72°C for 5 min.

RAPD analysis of *S. parauberis*

RAPD analysis was carried out by Romalde *et al.*, (1999) method. Six distinct random 10-mer primers (Pharmacia Biotech) were used, including primer P1 (GGTGCGGGAA), primer P2 (GTTTCGCTCC), primer P3 (GTAGACCCGT), primer P4 (AAGAGCCCGT), primer P5 (AACGCGCAAC), and primer P6 (CCCGTCAGCA). The amplifications were carried out in a PCR gradient thermal cycler (TaKaRa) with the following parameters: an initial denaturation step of 95°C for 5 min; 30 serial cycles of a denaturation step of 95°C for 1 min, annealing at 35°C for 1 min, and extension at 72°C for 2 min;
and a final extension step of 72°C for 5 min.

Computer data analysis

Images of the gels were analyzed using the NJTREE program of RAPDistance Ver. 2.00 (Saito and Nei, 1987). Computed similarities among isolates were estimated by means of the Phi coefficient Dendrogram clustering of the strains was based on the Neighbor-joining method.

Results and discussion

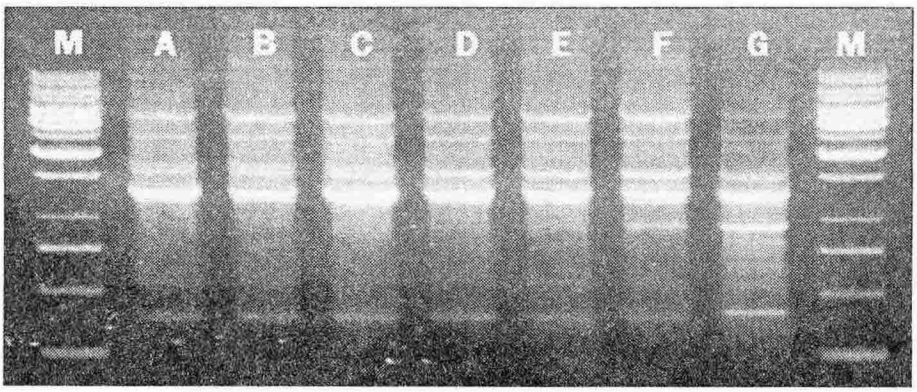

Fig. 1. RAPD fingerprints of *S. parauberis* strains employing the primer P1. Lanes: M: GeneRuler 1kb DNA ladder (Gibco); A: St-190; B: St-201; C: St-202; D: St-166; E: St-179; F: St-152; G: St-140; M: GeneRuler 1kb DNA ladder (Gibco).

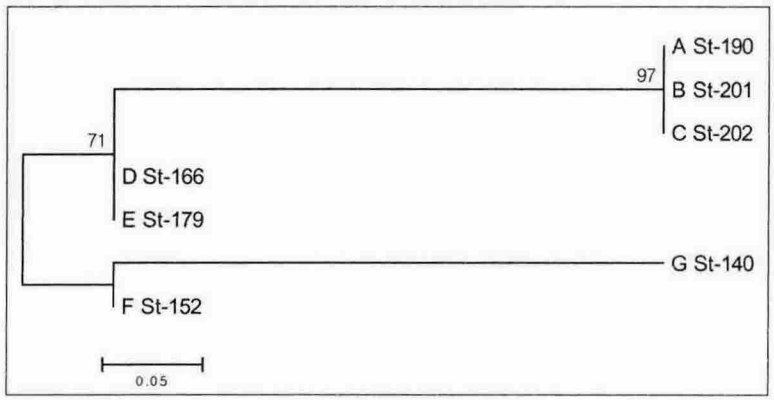

Fig. 2. Neighbor-joining tree based on analysis of RAPD fragment of *S. parauberis* strains.

Two infectious species of Streptococcosis pathogens were detected by multiplex PCR assay. Detection rates of *Streptococcus iniae* and *S. parauberis* could reach 44.9% and 55.1% respectively for one year during 2004 to 2005 in Jeju island. These findings showed that *S. parauberis* strains were important pathogen with streptococcosis of olive flounder in Jeju island.

In the present study we have investigated the interspecific relationship of all Jeju area of *S. parauberis* by RAPD analysis. Represent strains divided to four groups by RAPD fingerprints. The important differences observed between the olive flounder isolates suggest that they could constitute a well-differentiated group or a separate clonal line within this bacterial species. Though, serological research of *S. parauberis* strains in Jeju island not exist yet. These strains doing the serological evolution. Further studies including a higher number of isolates will confirm this hypothesis.

Sampling area of first clustering group strains were A, B and C area, beside wide distributed in Jeju island and second clustering group(D and E area) too. Third and fourth clustering group showed a independence distribution. These work may be an useful tool for epidemiological studies of this genetically heterogeneous *S. parauberis* isolates. In addition, knowledge of the geographical distribution of different genetic groups of this fish pathogen may be very helpful in designing preventive measures for effective control of strptococcosis, such as vaccine formulations.

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