African Journal of Microbiology Research Vol. 5(26), pp. 4722-4724, 16 November, 2011
Available online at http://www.academicjournals.org/AJMR
ISSN 1996-0808 ©2011 Academic Journals
DOI: 10.5897/AJMR11.674

Short Communication

Detection of *Streptococcus iniae* by polymerase chain reaction in rainbow trout (*Oncorhynchus mykiss*) in west Iran

Firooz Fadaeifard[1*], Mahdi raissy[1], Hasan Momtaz[2] and Reza Zahedi[3]

[1]Department of Aquatic Health and Disease, Shahrekord Branch, Islamic Azad University-Shahrekord-Iran.
[2]Department of Microbiology, Shahrekord Branch, Islamic Azad University-Shahrekord-Iran.
[3]Graduated of Shahrekord Branch, Islamic Azad University-Shahrekord-Iran.

Accepted 23 August, 2011

Streptococcosis is one of the serious diseases in cultured salmonids, specially rainbow trout, *Oncorhynchus mykiss*. *Streptococcus iniae* is one of the important pathogens for fish which is also dangerous for human. The aim of this study is to investigate the streptococcosis caused by *S. iniae* in rainbow trouts farms. For this purpose, we evaluate fishes that showed clinical signs similar to streptococcosis from 10 farms in west of Iran on the subject of *S. iniae* existence. At first, the specific culture for *streptococcus* was done and, then, polymerase chain reaction (PCR) for detection of *S. iniae* was used. Out of 50 evaluated specimens, 13 were suspected to *S. iniae* after biochemical tests. PCR uses specific primers for *S. iniae* and 6 samples were revealed positive. The results of this study showed the high existence of *S. iniae* in this region. PCR is an effective technique for the rapid and specific detection of *S. iniae* in fish tissues.

Key words: *Streptococcus iniae*, polymerase chain reaction (PCR), Chaharmahal –va- Bakhtyari Province, Iran.

INTRODUCTION

Streptococcosis is one the infectious systemic diseases, caused by organisms of the genus *Streptococcus*. The first streptococcal infection in cultured fish was reported in Japanese rainbow trout (Hoshina et al., 1958). This disease can be treated by antibiotics such as erythromycin, florfenicol and amoxicillin (Treves-Brown, 2000; Yanong et al., 2005). Several vaccines have been modelling and developed against streptococcal bacteria (Eldar et al., 1997; Shelby et al., 2002). *S. iniae* was isolated from ornamental cyprinid fishes (Russo et al., 2006). Different genera and species of gram-positive and catalase-ngative cocci are pathogenic to fish (Gittino et al., 2003). *S. iniae* type II infections can be identified by clinical and histopathological findings in rainbow trout (Lahav et al., 2004). This bacterium has emerged as an important fish pathogen in recent decades. The mortality rate caused by outbreak of this disease, in different fresh and seawater commercial fish species such as rainbow trout, tilapia, channel catfish, gilthead sea bream or sea bass, has been from 30 to 50%. (Shoemaker et al., 2001; Colorni et al., 2002; Eldar et al., 1995). Mata et al. (2004a) used the lactate oxidase-encoding gene (*lctO*) from mentioned bacteria as a target molecule for a PCR-based method by the aim of correcting and improving the detection and identification of this pathogen. Culture of salmonid has been increased in Iran in recent years in a way that majority of water source, including springs and rivers have been used for trout farming. One of the important economic region, with high production of rainbow trout, is located in west of Iran. By increasing of salmonids farming, the outbreak of infectious diseases, especially streptococcosis, was revealed. Therefore, in the present study, we try to identify the infected fishes by

*Corresponding author. E-mail: fadaeifard@gmail.com. Tel: 00983813361045.

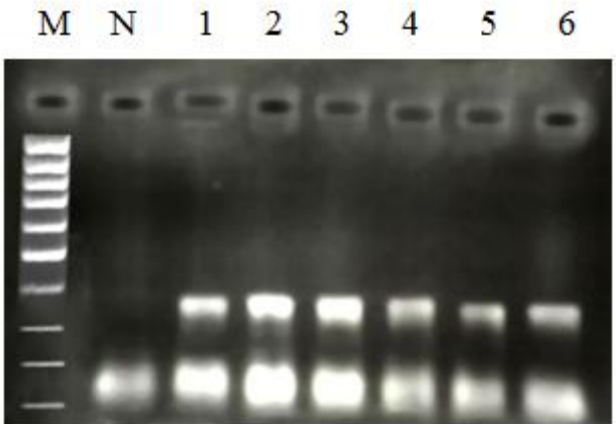

Figure 1. Presentative amplification products from infected rainbow trout using PCR assay for the detection of Streptococcus iniae lanes: M, 100 bp DNA Ladder, N: negative control, lanes 1-6 samples isolated from infected fishes.

PCR method in order to detect the *S. iniae* bacterium.

MATERIALS AND METHODS

Sampling and isolation of bacterium

During spring and winter 2008, sampling from 20 rainbow trout (*Oncorhynchus mykiss*) farms in Chaharmahal-va-Bakhtyari Province (located in west of Iran) were done. Infected fishes or suspected to diseases were randomly sampled from selected farms and then from some organs such as brain, kidney, spleen and liver, Sterile swabs were streaked on brain heart infusion agar plate (BHIA; Difco, USA) supplemented with 1.5% NaCl and plates were transferred to the lab beside the ice.

Isolation of pathogens and biochemical analysis

Plates transferred to the laboratory were incubated at 25°C for 48 h for growing the colonies. Single colonies from plates with pure culture growth were re-streaked on the BHIA media to obtain pure isolates. In each of the grown colonies, catalase test was done and Gram-positive cocci and catalase negative were sent for PCR test. In each step of PCR testing, distilled water as negative control is used.

DNA extracting

For isolation, pure colonies were put in tubes beside 100 microliter distilled water. DNA was extracted according to kit of extracting DNA (Sinagen Co, Iran).

Primers

The oligonucleotide primers that specifically amplified 373-bp fragments were used for PCR amplification, based on method of Berridge et al. (1998).

Strp-F (5'-GGAAAGAGACGCAGTGTCAAAAGAC-3') and Strep-R (5'-CTTACCTTAGCCCCAGTCTAACGAC-3').

V-PCR amplification test

The amplification reactions were performed in 50 µl reaction mixtures containing 0.1 mM of each dexoynucteotide, 15 pmol of each primer, 50 mM KCl, 10 mM Tris-Hcl (pH = 9), 2 mM $MgCl_2$,10%dimethyl sulfoxide (DMSO, Sigma), 1.5 U of Taq DNA polymerase (Sigma) and 40 ng of template DNA. The PCR reaction was carried out in a PCR programmed thermocycler (Eppendrof, Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany Co.) using the thermal profiles: initial cycle 95°C for 5 min, followed by a further 32 cycles: denaturation at 95°C for 60s; annealing at 60°C for 60s , and extension by polymerase at 72°C for 60 s.

RESULTS AND DISCUSSION

During this study, 100 samples of brain, kidney, spleen and liver were investigated, after receiving, the obtained samples, immediately they were cultured at brain heart infusion agar medium that results indicated that 13 isolates are *Streptococcus* sp., the Gram staining and catalase test were used for confirming primary isolated bacteria samples. Then 13 samples from suspected strains were isolated for molecular diagnosis and detection by PCR method, of which 6 cases were positive (Figure 1). After PCR, the 372 bp fragment obtained bands, were blasted with other sequences associated with *S. iniae* in the gene bank (NCBI, Gen Bank). The real identification of the used primers in determining the *streptococcus* species makes sure that all six species were *s. iniae*.

This study was carried out by the aim of molecular detection of *S. iniae* bacterium in farmed rainbow trout located in West of Iran that results to identification of these bacteria in the aforementioned fishes. This bacterium is the infectious agent of farmed fishes and the cause of high losses and mortality in different species of fishes. Therefore, preventing from spreading the diseases among different farms and controlling them is of great important. Brunt and Austin (2005) indicated that the use of probiotics can control the lactococcosis and streptococcosis in rainbow trout, as this immunostimolant materials add to feed result in stimulation of innate immunity, namely an increased number of leucocytes and enhanced phagocytic and respiratory burst activity.

Another fish pathogens that due to streptococcosis in rainbow trout is *Vagococus salmoninarum*. Molecular diagnostic tools, such as PCR assays, are increasingly used to detect and identify many different bacterial pathogens including the most significant fish pathogens such as *Yersinia ruckeri*, *lactococcus garvieae*, *Aeromonas salmonicida* (Blanco et al., 2002; Aoki et al., 2000). Ferguson et al. (1994); Nieto et al. (1995); Colorni et al. (2002) and Huchzermeyer et al. (2003) accomplished some studies about streptococcosis disease of fishes. These bacteria are not detectable

through the routine bacteriological methods.

Roach et al., (2006) during studies expressed that *S. iniae* were not detectable by most common bacterial systems. Thus PCR method was introduced as a confirmatory technique for certain identification of *S. iniae*. Multiplex PCR technique is one of the identification methods of fish through which can simultaneously detect multiple bacterial agents. And by this method increase the detection of few pathogens (Mata et al., 2004b), using these diagnostic methods succeed to detect the four species of *S. iniae, S. difficle, l. garvieae, Streptococcus parauberis* in pure culture and fish tissues homogenized solution.

Conclusion

In this study, isolation of *S. iniae* strains from rainbow trout farms at west of Iran and confirm with the PCR technique was done successfully which ultimately results to the identification of the said bacterium as a causative agent of mortality and lesions in fishes. Thus, because of increasing production of rainbow trout in ran's inland waters and the important role of this bacterium in pathogenicity and health risks for fish and human, next research on identifying the bacterial strains will be done genetically and phylogenically.

ACKNOWLEDGEMENT

This study was carried out in biotechnology research center in Islamic Azad University-Shahrekord branch. Authors thank all those who contributed to the success of this research work.

REFERENCES

Aoki T, Park CI, Yamashita H, Hirono I (2000). Speciesspecific polymerase chain reaction primers for *Lactococcus garvieae*. J. Fish Dis., 23: 1–6.

Berridge B, Fuller JD, De Azavedo J, Low DE, Bercovier H, Frelier PE (1998). Development of specific nested oligonucleotide PCR primers for the *S. iniae* 16S–23S ribosomal DNA intergenic spacer, J. Clin. Microbiol., 36(9): 2778–2781.

Blanco MM, Gibello A, Vela AI, Moreno MA, Dom´ınguez L, Fernández-Garayzábal JF (2002). PCR detection and PFGE DNA macrorestriction analyses of clinical isolates of *Pseudomonas anguilliseptica* from winter disease outbreaks in sea bream *Sparus aurata*. Dis. Aquatic Organ., 50: 19–27.

Brunt J, Austin B (2005). Use of a probiotic to control lactococcosis and streptococcosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum), J. Fish Dis., 28(12): 693-701.

Colorni A, Diamant A, Eldar A, Kvitt H, Zlotkin A (2002). *S. iniae* infections in Red Sea cage-cultured and wild fishes. Dis. Aquat. Org., 49: 165–170.

Eldar A, Horovitcz A, Bercovier H (1997). Development and efficacy of a vaccine against *S.iniae* infection in farmed rainbow trout. Vet. Immunol. Immunopathol., 56: 175–183.

Ferguson HW, Morales JA, Ostland VE (1994). Streptococcosis in aquarium fish. Dis. Aquat. Org., 19:1–6.

Gittino C, Latini M, Agnetti,Panzieri C, Lauro L, Ciappelloni R, Petracca G (2003).Emerging pathologies in aquaculture: Effects on productions and food safety. Vet. Res. Commun., 27: 471-479.

Hoshina T, Sano T, Morimoto Y (1958).A Streptococcus pathogenic to fish. J. Tokyo Univ. Fisheries, 44: 57-68.

Huchzermeyer KD (2003). Clinical and pathological observation on *Streptococcus* sp.infection on south Africa trout farms with gas supersaturated water supplies.onderstepoort. J. vet. Res., 70(2): 95-105.

Lahav D, Eyngor M, Hurvitz A, Ghittino C, Lublin A, Eldar A (2004). *S. iniae* type II infections in rainbow trout ,*Oncorhynchus mykiss*. Dis. Aquat. Organ., 62(1-2): 177-80.

Mata AI, Gibello A, Casamayor A, Blanco MM, Domínguez, L, Ferna´ndez-Garayza´bal JF (2004a). Multiplex PCR Assay for Detection of Bacterial Pathogens Associated with Warm-Water Streptococcosis in Fish, Appl. Environ. Microbiol., 70(5): 3183–3187.

Mata AI, Blanco MM, Domínguez L, Fernández-Garayzábal J, Gibello l (2004b). Development of a PCR assay for *S. iniae* based on the lactate oxidase (*lctO*) gene with potential diagnostic value. Vete. Microbiol., 101(2): 109-116.

Nieto JM, Devasa S, Quiroga A, Toranzo AE (1995). Pathology of Enterococcus sp. infection in farmed turbot, *Scophthalmus maximus* (L.). J. Fish Dis.,18: 21–30.

Roach JCM, Levett PN, Lavoie MC (2006).Identification of *S. iniae* by commercial bacterial identification systems. J. Microbiol. Methods 67: 20–26.

Russo R, Mitchell H, Yanong RPE (2006). Characterization of *S. iniae* isolated from ornamental cyprinid fishes and development of challenge models. Aquac., 256:105–110.

Shelby RA, Klesius PH, Shoemaker CA, Evans JJ (2002). Passive immunization of tilapia, *Oreochromis niloticus* (L.), with anti-*Streptococcus iniae* whole sera. J. Fish Dis., 25: 1–6.

Shoemaker CA, Klesius PH, Evans JJ (2001). Prevalence of *Streptococcus iniae* in tilapia, hybrid striped bass, and channel catfish on commercial fish farms in the United States. Am. J. Vet. Res., 62(2): 174–177.

Treves-Brown KM (2000).Applied fish pharmacology. Kluwer Academic Publishers, Dordrecht, The Netherlands, p. 294.

Yanong RPE, Curtis EW, Simmons R, Bhattaram VA, Gopalakrishnan VA, Ketabi N, Nagaraja NV, Derendorf H (2005). Pharmacokinetic studies of florfenicol in koi carp and threespot gourami *Trichogaster trichopterus* after oral and intramuscular treatment. J. Aquat. Anim. Health, 17: 129–137.