Isolation and Identification of Mycobacterium from Farmed Fish in Qazvin Province

Authors Name

Abstract

Aquatic tuberculosis is one of the common diseases between humans and aquatic animals in the world. Species of Mycobacteria cause tuberculosis in fish, the focus of these infections being water and can enter the human body through skin wounds and cause fish tank granuloma lesions. The purpose of this research was to isolate Mycobacterium from farmed fish and then to determine the molecular identification and genomic pattern of the isolates. 127 farmed fish were collected from 5 different pools and fish supply stores in separate areas of Qazvin. All samples were opened and cultured under microbial sterile conditions. Then, within a period of one to two weeks from bacterial culture and growth in dedicated medium, smeare was prepared from them, and stained with fluorochrome and Ziehl-Neelsen. The chromosomal DNAs of the bacteria were then extracted, followed by multiple PCRs of hsp65, IS6110, 16SrRNA and ITS for determination of species with specific primers. Also, 6 samples were digested enzymatically by RFLP using PVU II enzyme and hybridization was performed with DR, PGRS and Southern blotting markers. In the result of 127 farmed fish, 13 acid fast bacteria were isolated and all isolates were positive with Mycobacterium specific primer. The results of nucleotide sequencing reading were arranged with the Chromas and Clustal x program, which the isolates were confirmed with aquatic-specific Mycobacterium fortuitum and Mycobacterium marinum. Enzymatic digestion of 6 selected samples was performed well with PVU II enzyme and then with two specific probes DR and PGRS showed relatively good genetic diversity so that 2 genotypes were identified by DR probe and 5 genotypes by PGRS probe. This research provides strong evidence that Mycobacterium is present in Iranian farmed fish and is likely to be transmitted to humans and cause skin lesions.

Keywords: Mycobacterium Marinum; Mycobacterium Fortuitum; Fish Tank Granuloma; Farmed fish; Mycobacteriosis.

Introduction

Tuberculosis is one of the most dangerous bacterial infectious diseases that has both high transmissibility and high mortality and is often caused by poor health, poor living conditions and vitamin deficiency. Fish are at risk in crowded pools. Post-mortem examination reveals small white nodules in the internal organs. The cause of the disease is similar to other mycobacterial

animals. Different species of Mycobacteria cause tuberculosis (Cole et al., 1998). Fish mycobacteria is a chronic progressive disease. Fish in the fish ponds or in crowded aquariums are more susceptible to the disease. Some of the species of mycobacteria that commonly cause mycobacteria in ornamental fish include Mycobacteriti marinum, Mycobacterium chelonae, and Mycobacterium fortuitum. In addition, several other species of mycobacteria have been identified in relation to this disease (Pate et al., 2005). Mycobacteria that cause tuberculosis in fish are found everywhere, but most are found in soil, sewage, food waste and dead fish. The disease is most commonly caused by dead fish whose carcasses remain in their habitat long after they die. In addition to causing fish mortality, species of Mycobacterium can be transmitted to humans under certain conditions and cause skin infections called fish tank granuloma (Pourahmad et al., 2009). Non-tuberculosis mycobacteria such as Mycobacterium marinum can be transmitted from infected ornamental fish to the related medium (infected water) during contact with the aquarium during cleaning by handling fish through cuts or scratches on the skin and may result to human infection (Beran et al., 2006; Enzensberger et al., 2002; Jernigan and Farr, 2000; LeBlane et al., 2012; Lewis et al., 2003).

Mycobacterium chelonae and Mycobacterium fortuitum are fast-growing invasive species that are widely distributed in the medium (soil and water) and are common agents of nosocomial infections that usually cause superficial lesions and possibly primary pulmonary disease and lymphadenopathy (Kent Mishael et al., 2016; Mainous and Smith, 2005; Watral and Kent Michael, 2016; Woods et al., 2000). Diagnosis of this disease in farmed fish is usually done using histopathological methods, culture, bacterial growth characterization, pigment type, morphology of colonies and biochemical tests. Although the initial diagnosis of fish mycobacteria is based on external clinical signs and the presence of granuloma in visceral tissues by sampling, sometimes the fish have no clinical signs and no granuloma are observed (Gauthier and Rhodes, 2009). Aquaculture, including farmed fish, can serve as a source of exposure to human-transmitted pathogens. The aquaculture industry is growing rapidly and is potentially facing increasing numbers of fish farmers and processors. Reports of the incidence of such zoonotic infections acquired from farmed fish are not high, and these organisms have been identified as potential concerns. Diseases caused by opportunistic pathogens and the lack of effective treatment and the need for long-term treatment, in addition to economic languages caused by the adverse effects of fish mycobacteria such as reduced feed efficiency, reduced growth and increased loss, highlights the need for the identification of mycobacteria in farmed fish (Zanoni et al., 2008).

Materials and Methods

A) Sample Collection

In this research, which lasted from May 2013 to August 2014, 127 samples of farmed fish were selected from 5 fish farming pool and several fish supply stores in different parts of Qazvin. These samples were randomly selected from the loss population and were freshly frozen and transferred to the National Tuberculosis laboratory of razi Institute and all of them were opned and cultured separately under sterile condition in a few stages.

B) Ziehl-Neelsen Stain

Liver, spleen, kidney, intestine and various parts of the fish's body were separated using sterile scissors and cut into small sizes and poured into sterile falcone tubes containing sterile distilled water and pearl glass. They were homogenized in the vertex for 3 minutes. The sample was then filtered from current containers into new Falcone tubes by sterile gas inserted into the tubes and used for staining under Ziehl-Neelsen staining for acid fast bacteria. One hundred views were examined under a grid microscope at 1000 magnification at each prepared lams.

C) Bacterial Culture

After performing steps such as stripping, deinfection and homogenization, centrifugation and neutralizing the pH of the studied samples, the samples were cultured in Lowenstein-Jensen medium containing glycerin and adherent to the growth of Mycobacterium colony. After observation of the colony (s) after about 6 weeks of incubation, colonies were harvested for microscopic and molecular tests (Kane et al., 2007). In order to observe growth and to determine the growth rate of Mycobacteria, the culture media were examined daily for 4 weeks and then once a week for 3 months. Samples that their spread were positive but their cultures remained negative by the end of this period were incubated for another 4 weeks (In total, 6 weeks) (Kane et al., 2007; Moghim et al., 2012). The smears were also prepared from the colonies and two spread lams were prepared and stained for each (Fig. 1). Acid-fast bacilli were confirmed by fluorochrome and Ziehl-Neelsen stain. In this research, 13 acid fast isolates were seperated.
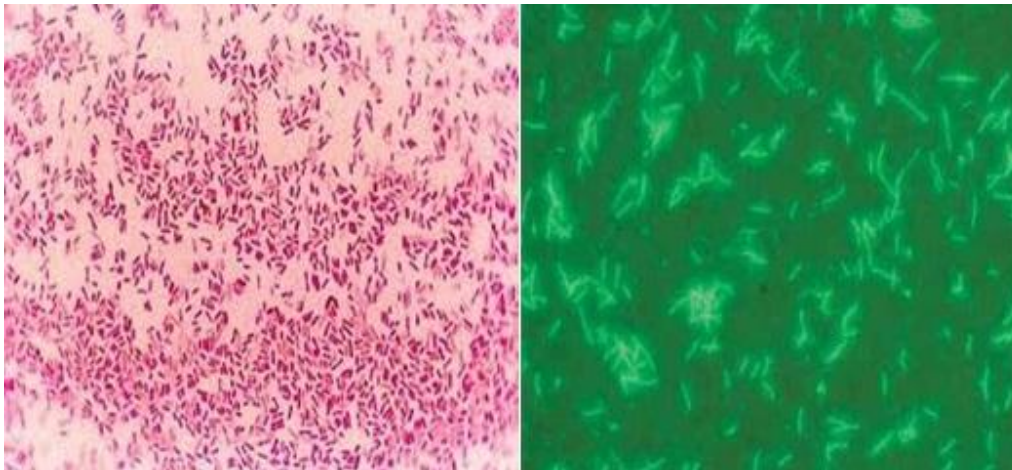
Fig. 1: Mycobacterial acid-fast bacilli in fluorochrome and Ziehl-Neelsen stains

D) DNA Extraction

At the phase of DNA extraction, at least one complete loop of bacterial colonies was harvested from the culture medium using a sterile loop under safe conditions and transferred slowly into a microtube containing 400 µl 1x TE buffer. The resulting suspension was placed for 30 min in 80 °C to inactivate the bacterial cells. Subsequently, the resulting suspension was centrifuged for 5 min and passed from filter 0.2. The filtered fluid contains genetic material for use in PCR tests (Van Soolingen et al., 2001), and genomic DNA was completely extracted without the slightest fracture and used for RFLP. For initial examination of the extracted DNA, electrophoresis was performed on agarose gel and subsequently, the amount and purity of DNA were determined by NanoDrop Spectrophotometer. Primers used to determine and identify Mycobacteria isolated from farme fish were listed in Table 1.

Table 1: Primers used determine and identify mycobacteria isolated from farmed fish

LOCUS	Nucleotide sequence
16S rRNA	F: 5'(ACGGTG GGTACTAGG TGTGGG TTTC) 3'
	R: 5'(TCTGCGATTACTAGCGACTCCGACTTCA) 3'
IS6110	INS-F 5'(CGTGAGGGCATCGAGGTGGC) 3'
	INS-R5' (GCGTAGGCGTCG GTGACAAA) 3'
HSP65	F: 5' (GCCAAGAAGACCGAYGACGT)3'

	R: 5' (GGTGATGACGCCCTCGTTGT)3'
ITS	F: 5' (GCTGGATCACCTCCTTCT)3'
	R: 5' (CTGGTGCCAAGGCATCCA)3'

Molecular Identification of Isolates

In this research, the SrRNA 10/S6 16 and RD typing locus (RD12 RD RD4 and RD) were used to perform the PCR test (9). For PCR, a commercial mixture called A ®mpliquon was used and the final volume of each reaction was adjusted to 16 μl. Finally, the PCR product was electrophoresed. Temperature, time and cycle of the thermocycler were tested in PCR to confirm the composition type of the Mycobacterium isolates with 16SrRNA, IS6110 and RD typing locus (Warren et al., 2006).

Enzymatic Digestion of Chromosomal DNA and DNA Fragmentation

Restriction endonucleases are enzymes that specifically identify and cut a particular sequence. The endonuclease restriction enzyme recognizes short DNA sequences and cuts at specific locations within or adjacent to the identified double-stranded DNA sequence. This causes the cuts of DNA fragmentation. The Pvu II enzyme creates cuts in the CAG/CTG sequences. In this research, isolates of Mycobacterium tuberculosis were digested with Pvu II enzyme and then all electrophoresis, membrane transfection, hybridization with PGRS and DR probes were performed separately and detection was performed on them. The method of digestion of chromosomal DNA with Pvu II restriction enzyme is that at the beginning of the work, 2 mg of DNA samples were evaluated using a nanodrop machine. Prior to enzymatic digestion, the precise amount of DNA was determined and approximately 2 μg of Mycobacterium bovis AN5 genomic DNA was transferred to a final volume of 20 μL in a microtube and digested. To isolate DNA fragments with molecular weights of about 0.5 to 25 kl in agarose gel for analytical and preparatory purposes, digested DNA was electrophoresed and then transferred to the positively charged nylon membrane (Southern blotting). All the conditions and requirements mentioned above were met.

Southern Blotting

In general, Southern blotting in molecular genetics refered to the transfer of molecules separated

by electrophoresis from a gel to a specific membrane. In southern blotting technique, electrophoretic transfer of isolated DNA fragments (with negative charge due to phosphate bands) to a positively charged nylon membrane for use in hybridization operations using digoxigenin-labeled probes were performed. Subsequently, the detection and identification of hybrid probes is carried out using Alkaline Phosphatase (AP) conjugated digoxigenin antibody and NBT and BCIP substrates.

Southern blotting is done in two ways including capillary transfer and vacuum transfer. Vacuum transfer is much faster than capillary transfer (about 1-2 hours) but the capillary method requires less equipment and is more convenient and the transfer quality is also better. In this research, due to the availability of all the equipment needed for capillary transfer in the tuberculosis department, this method was used. At this stage, due to the use of ethidium bromide and UV radiation, all precautions mentioned in the previous steps were followed. Before blotting, the gel is subjected to UV irradiation and treated with HCl to allow uniform DNA transfer to the membrane. Acid treatment allows to create the relative deposition. The deuterated sites are cutten alkaline during treatment and this enables the DNA fragments to be separated to prepare single-stranded DNA after transfer to the membrane for probe hybridization. In the capillary transfer method, the DNA molecules on the agarose gel move with the buffer flow capillary effect and precipitate on the adsorbent nylon membrane which has a positive electric charge and rests on the solid surface of the substrate immediately after the gel. Agarose gel is very unstable for studying DNA strands that contain information. In this method, the liquid flow is generated by the capillary activity of the adsorbent membranes, which are placed on the surface of the gel and the nylon membrane. Subsequently, the solutions below the gel surface move within the gel and the membrane and are absorbed by the absorbent paper on the membrane. Following the movement of these fluids, the DNA fragments are transported by the liquid flow and exhaled from the gel and bound to the membrane, precisely where the DNA is located on the gel. Charged nylon membranes are more commonly used today. These membranes carry amine groups (NH4) and are known as positively charged nylons. Single-stranded or double-stranded nucleic acids bound from the back bone region to these membranes. The purpose of this method is to form an image of agarose gel contents on the surface of the membrane in a straightforward manner. The transferred DNA is then fixed on the membrane in various ways that covalently bound the DNA to the membrane. Such DNA is stable in its location and can react with probes or other materials. The end result is that the reactive DNA on the membrane is spatially similar to the DNA on the original gel.

It should be noted that the DNA charge is negative due to the presence of phosphate and the nylon membrane used must be positively charged and this charge difference not only facilitates the transfer but is essential for proper transfer. In part of this research, nylon-free membranes were used and it was found that DNA transfer was not performed well without positive charge of the membrane. In vacuum transfer, the gel is in contact with a filter support on a porous grid above a vacuum chamber. The buffer is pulled high from a tank and the nucleic acids are washed from the gel and deposited on the membrane. Vacuum transfer is much faster than capillary transfer.

Hybridization Probes

The probes are fragments of single-stranded DNA or RNA molecules that are labeled in different ways, and these labeled fragments are used as probes to hybridize with their nucleotide sequences complementary to the target DNA, under specific laboratory conditions. The hybridized regions were then sequenced in different ways to reveal the differences of the strains in terms of probe replacement in specific chromosome regions. The probes are generally divided into three major groups including oligoprobes (such as PGRS and DR), PCR probes (such as IS6110 and IS1081) and RNA probes. In this research, 2 oligoprobes PGRS and DR were used.

Polymorphic GC-Rich Repetitive Sequence (PGRS) Probe

The digoxigenin-labeled PGRS oligonucleotide used in this research was ordered by Tuba-Negin Company and it was obtained from DNA Technology A/S Company, Denmark. The microtubule containing the probe that was lyophilized was dissolved in 500 µL of sterile distilled water until its final concentration reached 0.158 Pmol/µl. The probe was then divided into several microtubes and stored at -20 °C for use in subsequent steps. The sequence of this probe is as follows.

5' CGG CCG TTG CCG CCG TTG CCG CCG TTG CCG CCG3'

There are three methods to label oligonucleotides with digoxigenin:

1-3'end labeling   2-3' tailing   3-5' end labeling

In this paper, digoxigenin-labeled PGRS and DR were used by the first (3'end labeling) method, made by DNA Technology A/S Company, Denmark.

Pre-Hybridization and Digoxigenin-Labeled Probe Hybridization

Pre-Hybridization Phase

The pre-hybridization phase prepares the membrane for the hybridization phase by covering the non-specific portions binding with nucleic acid, thereby minimizing the background black color. The procedure was that the membrane was tubed without folding and placed in a special tube of hybridization oven and the pre-hybridization solution was poured into the tube. (The volume of solution is equal to 20 ml per 100 cm^2 of the membrane surface). The tube was then inserted into the hybridization oven and stored at 65 °C for 3-4 hours.

Hybridization Phase

After completing the pre-hybridization phase, the pre-hybridization solution was removed from the hybridization oven tube and discarded, and the steps were carried out as follows. The hybridization solution containing digoxigenin-labeled PGRS probe was poured into the tube to the same extent as the pre-hybridization solution used in the previous step (Fig. 2). Labeled probe with appropriate concentration and appropriate amount was removed according to the volume or in other words the membrane area with the sampler and added to the hybridization solution (In this test, 10 ml of probe was used for 50 ml hybridization buffer). The optimum hybridization temperature was set for the probe, which was determined by the length of the probe and the size of the sequence corresponding to the target sequence (homology). In this research, hybridization was performed for a night at 65 °C. The membrane was then washed as follows to remove unbound probes, otherwise the black in background would be too high.
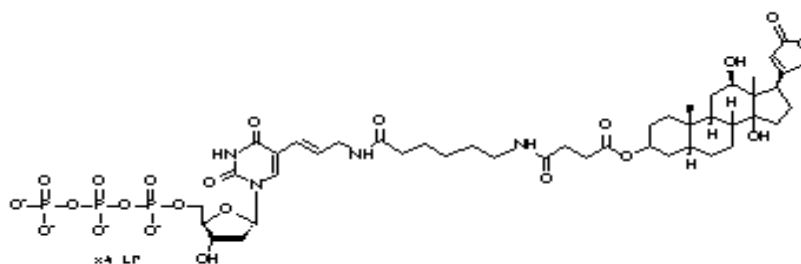

Fig. 2: Digoxigenin

Findings

PCR-16SrRNA Test:

By performing PCR on acid fast isolates, belonging all sample isolated from fish to Mycobacterium were confirmed. Applying this strategy to all isolates resulted in the observation of a specific electrophoretic band of 546 base pair (bp) on agarose gel (Fig. 3).
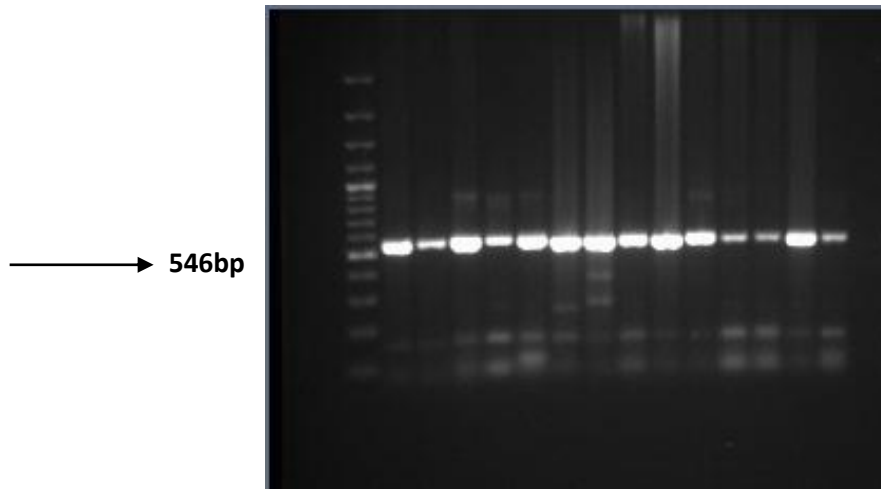

Fig. 3: PCR product electrophoresis for 546 bp of 16SrRNA sequence

M: size marker, +: positive control, columns 1 to 13: the studied fish isolates, -: negative control

PCR-IS6110 Test:

By PCR test on the samples, the belonging of none of the isolates to Mycobacterium tuberculosis complex were specified. In applying this technique to the isolates, no specific electrophoretic band of 245 bp was observed on agarose gel (Fig. 4).
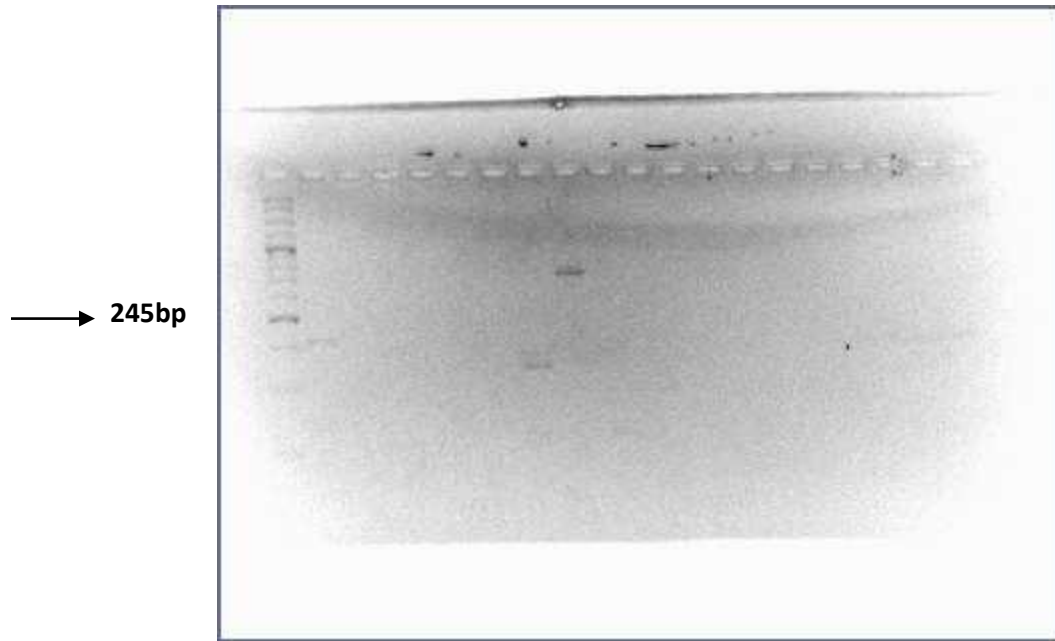
Fig. 4: PCR product electrophoresis for 245 bp of IS6110 sequence

M: size marker, +: negative control, columns 1 to 16: the studied fish isolates except column 8 which (-) is positivecontrol.

PCR-HSP65 Test:

By performing PCR on samples, belonging all sample to Mycobacterium were confirmed. Applying this strategy to all isolates resulted in the observation of a specific electrophoretic band of 295 base pair (bp) on agarose gel (Fig. 5).
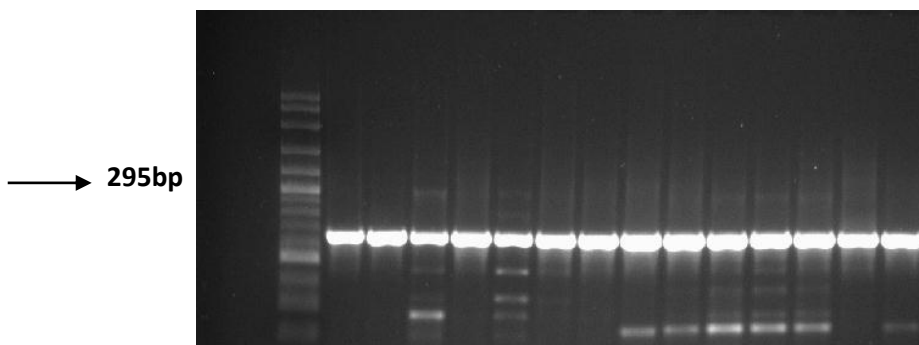
14M 1 2 3 4 5 6 7 8 9 10 11 12 13

Fig. 5: PCR product electrophoresis for 295 bp of Hsp65

M: size marker, in columns 1 to 13 of the studied fish isolates, final column: positive control

ITSPCR Test:

Applying this strategy to all isolates resulted in the observation of a specific electrophoretic band of 380 base pair (bp) on agarose gel (Fig. 6).
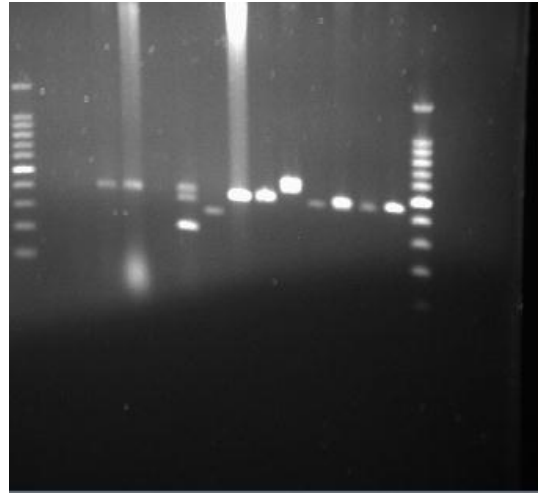
Fig. 6: M: marker size on both sides, column No. 1: the studied sample, column No. 2: Negative control, columns 3 to 14: the studied samples

Sequencing and Alignment

All 13 Mycobacterium isolates obtained were prepared for nucleotide sequencing. The isolates were sent to Macrogen Company, Korea and finally all 13 samples were sequenced with the hsp65, 16SrRNA primer and ITS by double reading of the nucleotides. By using blast n software and comparing the sequences sorted with a large number of Mycobacterium marinum and Mycobacterium fortuitum strains registered in the gene bank, the belonging these two samples to Mycobacterium marinum and Mycobacterium fortuitum were confirmed.

RFLP Test Based on PGRS and DR Markers:

Six isolates with standard strain of AN5 were used as a control for genomic fingerprinting and polymorphism of Mycobacterium tuberculosis isolates from fishes of Qazvin. For technical reasons and the quality of the extracted genomic material, 6 DNA samples were extracted from these isolates was appropriately diagnosed to perform this technique (Fig. 7).

1 2 3 4 5 6 7 8 M   M 8 7 6 5 4 3 2 1

23130bp

11755bp
11144bp
9988bp

7460bp

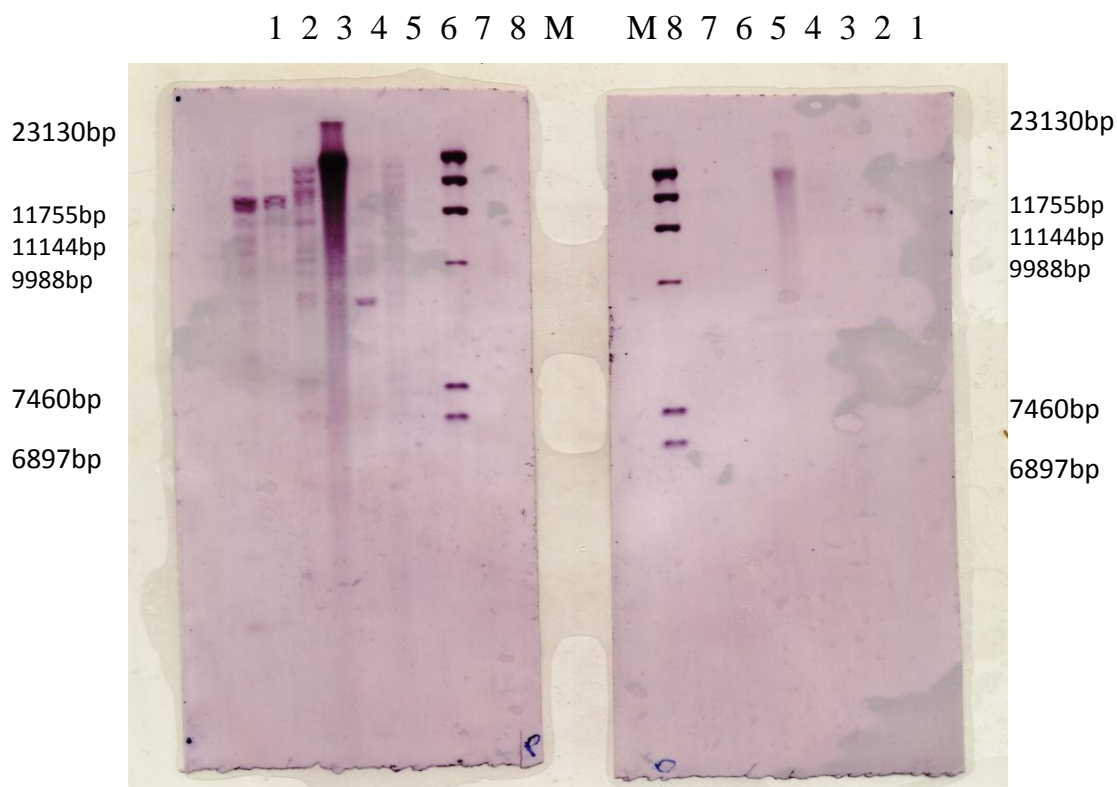6897bp

23130bp

11755bp
11144bp
9988bp

7460bp

6897bp

Fig. 7: RFLP with Pvu II enzyme and hybridization with PGRS and DR probes

The above investigation indicates that the similarity between the strains of Mycobacterium tuberculosis complex and the strains isolated from fish was very rare and there was a significant difference compared to standard human tuberculosis strains. In this research, 5 different genomic patterns were obtained that indicate that 5 strains were probably circulating among the mycobacteria isolated from farmed fish in Qazvin and these genomic patterns had single bands which analyzed with Gel Pro software due to the lack of similar genomic pattern in Iran.

Discussion

According to the OIE s and the World Health Organization (WHO) guideline, culture and isolation of Mycobacterium is the most definitive method of diagnosing tuberculosis (Epizooties, 2004). In this research, because of the unknown bacterial strains, cultures in Lowenstein-Jensen medium with glycerin, Pyruvate, mycobacterial-free and mycobacterial Herrold's Egg for early isolation of Mycobacterium from farmed fish. In the standard method for isolation of Mycobacterium, Lowenstein-Jensen medium have been introduced as the best mediums (Epizooties, 2004). Although sample culture is a definitive test for confirmation of mycobacterial infections, it may take weeks or months (Ayele et al., 2001). Mycobacteria that are present in various animals, such

as aquatic animals, are easily transmitted to humans and are highly pathogenic and can cause disturbing diseases in humans. Considering the investigations in the present research and the fact that Mycobacterium hammers can be isolated from various sources and capable of infecting a wide range of human and animal organisms, these bacteria can be considered as one of the most important of country's health and economic problems.

In 2008, in a descriptive study, 62 HIV-positive patients were randomly selected from patients referred to an addiction treatment center in Ahvaz by TST test with PPD 5 and IgM assay against bacillary TB antigens. This research showed that the prevalence of latent TB infection among HIV-positive injecting addicts in the studied area is higher than in other parts of the world. Tuberculin skin test is a useful test for the diagnosis of latent tuberculosis in HIV-positive people and is superior to the IgM assay against Mycobacterium tuberculosis antigens. The incidence of these mycobacteria is higher in people with immune deficiency or weakened immune systems, such as the elderly, children, and patients with pulmonary problems, which can spread tuberculosis and affect many organs.

In 1994, in a study by Wayne and Kubic (1999), the water distribution system was also identified as an important source of infection in hospitals, factories, and commercial and residential buildings (Wayne and Kubica, 1986). Searches and investigations that are useful through DNA and PCR tests for the presence of pathogenic bacteria in the environment, various body tissues and fish eggs. DNA-based methods, such as randomly amplified polymorphic DNA and pulsed-field gel electrophoresis of DNA, which are important methods for detecting genetic interactions between pathogens isolated from different geographic regions, have been efficient and used in epidemiologic research. The major antimicrobial drugs used in aquaculture are synthetic antibiotics and compounds such as sulfonamides and nitrofurans (Decostere et al., 2004). Species of peripheral mycobacteria in sediments of fish farming pools of northern Iran were investigated by Saeedi et al. After sampling from different areas and pools, cultures were performed only in Lowenstein-Jensen medium and at 37 °C which only due to the biochemical properties, several species of Mycobacteria were identified, the most abundant being Mycobacterium fortuitum.

In another research, the frequency of fish tank granulomas and Mycobacterium marinum were investigated in the fishery industry of Golestan province, which a clinical examination of 387 fishery personnel contacting fish was conducted for cutaneous wounds (Ghazi and Mohammdadi, 2009; Roberts, 1989). In another study conducted by Dr.Alaien et al. in Arak, a granuloma in 25-year-old woman finger was reported to be working at an ornamental fish storage facility in Khomein, who had a similar lesion in a patient colleague. The results of that research showed that

45% of infections occurred in fish-related occupations or water-related leisure and entertainment activities, and in 61% reported a history of trauma (Rastogi and McFadden, 1992).

The present research, after collecting the sample, fish head and tail and viscera was separated for the disinfection examination and due to the possibility of greater infection, the normal soda was used as a strong disinfectant. After microbial culture under sterile conditions, the samples were incubated at 25 and 37 °C. Bacterial colony growth was present in all four mediums, but because Lowenstein-Jensen medium is the standard Mycobacterium specific medium, bacterial growth in this medium was significantly greater than that of Herrold's Egg and Mycobacterium Herrold's Egg. A smear was prepared to identify the isolates from farmed fish. Genomic DNA was extracted after identifying of the acid fast being of bacteria, and 16S rRNA specific primers were used to ensure that the isolates were Mycobacterium. One PCR was performed with IS6110 primers to identify TB complexes, of which 13 isolates were not positive to this strategy. Given that the isolates studied in farmed fish are directly related to nutrition and public health and most of the samples in the previous studies include Mycobacterium marinum, and Mycobacterium fortuitum, further studies on the presence of Mycobacterium tuberculosis complex in fish are necessary. After confirmation of mycobacterium being of isolates, PCR was performed using aquatic-specific mycobacterium marinum primers, two of which were Mycobacterium marinum. Subsequently, several PCRs were performed using the primers of the heat shock protein of HSP65 that identify many mycobacteria.

RFLP technique and then Southern blotting technique were used to differentiate 13 strains isolated from fish. In the RFLP technique, Pvu II enzyme was used to digest the enzyme developed by Van Soolingen (2002) for the genomic fingerprinting of Mycobacteria, and 5 genotypes were identified using PGRS probe and two genotypes with DR probe. Due to the large size of the mycobacteria genome, it is difficult to interpret the RFLP pattern alone without hybridization, whereas after DNA transfer to the membrane and hybridization with probe and detection, interpretation is possible. Given that no genomic fingerprinting has been done in aquatic species, the results of this research suggest that specific probes are needed to determine the genomic pattern of Mycobacterium in aquatic species, and it is difficult to interpret the results with the two PGRS and DR probes most commonly used for Mycobacterium tuberculosis and are likely to be mistaken. Five genomic patterns obtained in this research were not similar to the standard strains of Mycobacterium tuberculosis. Due to the importance of Mycobacterium pathogenicity in farmed fish and in Iran, no so far coherent research has been performed in this field. In this research, several PCRs were performed using 16SrRNA and hsp65 primers. All 13 sample isolates were

selected for sequencing and sent to Korea to coordinate nucleotide sequencing, with coordination between Razi Institute and Macrogen Company. Finally, after nucleotide reading, all 13 samples were sequenced with the HSP65, 16S rRNA and ITS primers.

This research showed that the best method for identifying Mycobacterium strains is nucleotide sequencing, as it makes it possible to compare the desired strain with other similar strains, and the species affinity can also be easily estimated with Blast n and does not need to use different primers. This research also made it clear that the results of different PCR tests for strains are not always true and, unexpectedly, many of these tests are not specific and that infection in these tests will always present which alter the results and may as with this research, there is little contradiction with nucleotide sequencing. It is noteworthy that two Mycobacterium marinum were isolated in this research due to the infection of the isolated samples from the farmed fish and this strain is the causative agent of fish tank granuloma, and since the presence of granuloma in people's hands and feet and the confirmation of acid fast bacilli by staining lams has been reported in Iran country so far, it is a strong reason for the presence of Mycobacterium marinum and it requires further research. As well as the introduction of infected fish into the human food chain and its relationship to nutrition, it requires serious and extensive research across the country.

Conclusions

In recent years, a great deal of attention has been paid to mycobacterial infections in farmed fish due to increased fish production worldwide and greater attention to public health. In this research, some isolated bacteria such as Mycobacterium marinum, Mycobacterium fortuitum, and Mycobacterium smegmatis, which are known pathogens in fish and humans, were isolated from farmed fish. It has also been shown that isolated strains are resistant to most of the drugs used in treatment, given the zoonosis and difficulty of treatment and the need for long-term treatment, those with aquaculture equipment, particularly in individuals with immunodeficiency, greater consideration should be given to the maintenance of farmed fish to prevent and reduce the transmission of disease to humans. As a result, the research provides strong evidence that Mycobacterium is present in Iranian farmed fish and is likely to be transmitted to humans and cause skin lesions.

Acknowledgments

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