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Original Article

Serotypes, virulence genes and polymorphism of capsule gene cluster in *Lactococcus garvieae* isolated from diseased rainbow trout (*Oncorhynchus mykiss*) and mugger crocodile (*Crocodylus palustris*) in Iran

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Abstract

Background: *Lactococcus garvieae* causes lactococcosis in rainbow trout in many parts of the world. **Aims:** This study was conducted for the existence of the virulent factors and differentiation of the two serotypes in *L. garvieae*. **Methods:** Twenty-two strains of *L. garvieae* isolated from diseased rainbow trout from farms in different regions and mugger crocodile of Iran, were investigated. In order to rapidly detect the presence of the *hly1*, *hly2*, *hly3*, *NADH oxidase*, *sod*, *pgm*, *adhPsaA*, *eno*, *LPxTG-3*, *adhCI*, and *adhCII* virulence genes, two multiplex polymerase chain reaction (PCR) assays were developed. Also, simplex PCR method was used to identify the bacterial serotypes, *CGC*, *LPxTG-2*, *Adhesion*, and *adhPav* virulence genes using the specific primer. **Results:** All varieties of *L. garvieae* contained the *hly1*, *hly2*, *hly3*, *NADH oxidase*, *pgm*, *adhPav*, *LPxTG-3*, *sod*, *eno*, *adhPsaA*, *adhCI*, and *CGC* virulence genes. Also, *adhCII* gene was present in all strains except one of the isolates originated from mugger crocodile. In addition, *LPxTG-2* gene was only present in one of the isolates belonging to mugger crocodile. *Adhesion* gene was not present in all the strains. Interestingly, all the 22 strains originated from both hosts were identified as belonging to the serotype I. Based on the phylogenetic sequences of the capsule gene cluster, group all fish isolates into a cluster together with one isolate obtained from mugger crocodile. **Conclusion:** Further studies are recommended to investigate the role of virulence genes in *L. garvieae* and evaluate their pathogenicity to rainbow trout.

Key words: Iran, *Lactococcus garvieae*, Rainbow trout, Serotype, Virulence factors

Introduction

Lactococcosis is caused by a pathogen called *Lactococcus garvieae*. These bacteria cause hyper-acute and hemorrhagic septicemia in both wild and farmed fish species in seawater and freshwater related to activities concerned with the rearing of aquatic animals, especially in situations where the increase in the temperature for water exceeds 15°C during the summer. The clinical effects of the disease in the trout farming lead to considerable economic losses around the world (Meyburgh *et al*., 2017), including Iran (Akhlaghi and Keshavarzi, 2002; Karsidani *et al*., 2010; Sharifiyazdi *et al*., 2010; Erfanmanesh *et al*., 2012).

Despite the importance of this bacterium in the Iranian farms, the presence of genetic diversity and virulence genes among *L. garvieae* strains has not been studied sufficiently. Several researchers have suggested that one of the virulence genes for fish is the formation of a capsule in bacteria (Yoshida *et al*., 1997). The capsule, by inhibiting opsonophagocytosis and host serum killing activity, enhances the ability of the bacteria to withstand phagocytosis (Ooyama *et al*., 2002). Unexpectedly, uncapsulated strains of *L. garvieae* Lgper and the ATCC 49156 acted as causative agents for rainbow trout and the mortality rate they generated exceeded 89%. Therefore, it is unlikely that the capsule formation be an essential virulence determinant (Türe *et al*., 2014). Therefore, it seems necessary for the researchers to detect virulence factors in different strains of *L. garvieae*.

Two phenotypes of *L. garvieae* are referred to as agglutinating (KG+) and non-agglutinating (KG−) which are isolated from yellowtail (*Japanese amberjack*) (Ture and Altinok, 2016). In yellowtail, capsulated (KG−) Lg2 strain is more mortal than the non-capsulated ATCC 49156 strains (Morita *et al*., 2011). Slide agglutinating test with antiserum is a simple and rapid method for the diagnosis of agglutination (Fukuda *et al*., 2015).

The present study was aimed to compare the serotypes, virulence characteristics, and phylogenetic lineage of strains obtained from diseased rainbow trout and mugger crocodile in Iran.

Materials and Methods

Bacterial isolates

Two hundred rainbow trout likely to have streptococcosis/lactococcosis were collected from local farms in Tehran, Mazandaran, Kohkiluyeh and Boyer-Ahmad, Fars, Charmohal-va-Bakhteyari and Lorestan provinces of Iran between January 2017 and September

2018. They were tested for the existence of the etiologic agent that caused the disease. Totally, 20 *L. garvieae* isolates from diseased fish were studied. In addition, two samples were obtained from the oral cavity of mugger crocodile captured on two places in Negor protected area in Southeast of Iran near Chabahar County in Sistan and Balouchestan province.

DNA preparation and molecular characterization of *L. garvieae*

The boiling method for the extraction of bacterial genomic DNA was used (Englen and Kelley, 2000). The molecular characterization of *L. garvieae* isolates was accomplished by a species-specific polymerase chain reaction (PCR) assay based on *16S rDNA* sequence (Fig. 1) (Mata *et al*., 2004).

Primers

A rapid PCR assay was employed for the definitive differentiation of serotypes I and II in *L. garvieae* based on the differences in the PCR product sizes (Ohbayashi *et al*., 2017). Virulence genes were investigated in all *L. garvieae* isolates according to instructions offered by Miyauchi *et al*. (2012). The details of the primers which were employed in the current research are presented in Table 1.

PCR assays used for detecting virulence factors

Each of the PCR reaction mixes (in total 20 µL) contained 2 µL of DNA sample, 1 µL of each primer, 10 µL of 2× Master Mix PCR mixture (Ampliqon, Denmark), and 6 µL of distilled water. The process of amplifying the DNA was done in a thermocycler (Bio-Rad) following the procedure described here: a 7 min initial denaturation at 95°C; then, 34 cycles of denaturation at 94°C for 50 s, followed by annealing at

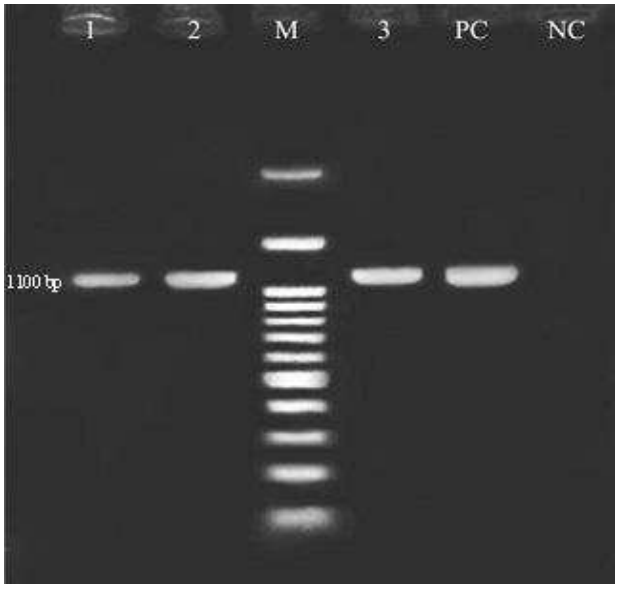

Fig. 1: Electerophoretic analysis (1.2% agarose gel) of amplified fragments (1100 bp) of *16S rDNA* sequences, which is specific for *L. garvieae* from 3 isolates in this study compared with the standard strain. M: DNA ladder (100 bp), Lanes 1 to 3: Positive samples of *L. garvieae*, PC: Positive control, and NC: Negative control

Table 1: Virulence genes: primer sequences, target genes, locus tag, amplified product size, and annealing temperature

Primer	Primer sequence (5´-3´)	Target gene	Locus	Product size (bp)	Annealing temp. (°C)
H1-F	CCTCCTCCGACTAGGAACCA	*Hemolysin 1*	LCGL 0323	521	58.6
H1-R	GAAAAGCCAGCTTCTCGTGC				
H2-F	TCTCGTGCACACCGATGAAA	*Hemolysin 2*	LCGL 0374	492	58.6
H2-R	TGAACTTCGGCTTCTGCGAT				
H3-F	AACGCGAGAACAGGCAAAAC	*Hemolysin 3*	LCGL 0597	291	58.6
H3-R	CCCACGTCGAGAGCATAGAC				
NADHO-F	TGCGATGGGTTCAAGACCAA	*NADH oxidase*	LCGL 0664	331	58.6
NADHO-R	GCCTTTAAAAGCCTCGGCAG				
SOD-F	GCAGCGATTGAAAAACACCCA	*Superoxide dismutase*	LCGL 0664	80	58.6
SOD-R	TCTTCTGGCAAACGGTCCAA				
PG-F	AAGTTTACGGCGAAGACGGT	*Phosphoglucomutase*	LCGL 0285	997	58.6
PG-R	TTTTCTGGTGCATTGGCACG				
AP-F	TGCCATGAAGATTGCACTGGT	*Adhesin Pav*	LCGL 1330	125	58.6
AP-R	GGTATTGATTTTGCGAAAACCCG				
APSA-F	GTTGCAACAGCTGGACACAG	*Adhesin PsaA*	LCGL 1533	180	58.6
APSA-R	ATACGGTTGAGTTGGGCTGG				
E-F	CAAGAGCGATCATTGCACGG	*Enolase*	LCGL 1514	201	58.6
E-R	CATTCGGACGCGGTATGGTA				
LP2-F	GCCAGTGAGAGAACCGTTGA	*LPxTG-2*	LCGL 1410	767	58.6
LP2-R	CAGGTTCAAGTGCAACTGCC				
LP3-F	TTAAGCACAACGGCAACAGC	*LPxTG-3*	LCGL 1585	231	58.6
LP3-R	CACGCGAAATGATGGTGCAT				
AC1-F	TTGGGCACATCAGACTGGAC	*Adhesin cluster 1*	LCGL 0842	264	58.6
AC1-R	AGCATCATCAGCTGCCAAGT				
AC2-F	CTGCGAGTGGCATCTCCATT	*Adhesin cluster 2*	LCGL 0843	160	58.6
AC2-R	TCAACACTGCGACCTTCTGT				
AF-F	CAGCCAGCACCAGGTTATGA	*Adhesin*	LCGL 0196	358	58.6
AF-R	CTCCTGCGTTGACATGGACT				
CGC-F	CTATGGCATTAGTCAGGAAG	*Capsule gene cluster*	LCGL 0431-LCGL 0448	744	58.6
CGC-R	GCTGTCATCATATTGTGTCCA				

58.6°C for 1 min, extension at 72°C for 60 s; and final extension at 72°C for 7 min. PCR mixture for the controls contained:
(1) No DNA template (reagent control)
(2) DNA from *L. garvieae* using *L. garvieae*-specific primer (positive control)

Following the PCR, 7 µL of PCR products were used for electrophoresis in 1.5% (w/v) agarose gel made ready with 1× Tris-borate-EDTA (TBE) buffer and run at 100 V for 60 min. Afterward, RedSafe (Intron Biotechnology, Seongnam, South Korea) was used to stain the DNA bands and they were next viewed by UV transillumination. In order to determine the sizes of the PCR products, they were compared with the migration of a 100 bp DNA ladder (K-Plus DNA Ladder, SinaClon, Tehran, Iran). DNA sequencing was also used for the confirmation of all the 13 virulence gene PCR products.

Multiplex PCR assays for the detection of eleven virulence genes

In order to reach the optimal specificity and sensitivity in the multiplex PCR assay, various annealing temperatures were tried. Both the intensity of the amplicons for each target DNA and the absence of nonspecific bands were considered in determining the optimal multiplex PCR conditions. Each 40 µL multiplex PCR reaction mixture (prepared on ice) contained 5 µL of the sample DNA, 20 µL of 2× Master Mix PCR mixture (Ampliqon, Denmark), 0.5 µL (*NADH oxidase* and *hly3*), 0.7 µL (*LPxTG-3* and *adhPsaA*) and 0.9 µL (*hly2* and *pgm*) of each primer and 6.6 µL of distilled water for 6 genes and 5 µL of the sample DNA, 20 µL of 2× Master Mix PCR mixture (Ampliqon, Denmark), and 0.3 µL (*adhCI*), 0.9 µL (*sod*, *adhCII*, *eno*, and *hly1*) of each primer and 7.2 µL of distilled water for five genes.

A Bio-Rad PCR machine (USA) was employed to do the thermal cycling as described here: an initial denaturation cycle at 95°C for 7 min; then, 34 cycles of amplification (denaturation at 94°C for 50 s, annealing at 56.8°C for 60 s, and extension at 72°C for 60 s), and a final 7 min extension period at 72°C. Controls consisted of the PCR mixture containing:
(1) No DNA template (reagent control)
(2) DNA from *L. lactis* (negative control)
or (3) DNA from *L. garvieae* using specific *L. garvieae primer* (positive control)
Following the PCR, the products were transferred to a 2.8% agarose gel and electrophoresed; and RedSafe staining was used for the visualization of the DNA.

DNA sequencing

In order to make sure that the amplification of the right products had taken place, from each gene, PCR product was sequenced directly (Macrogen Inc., South Korea). Furthermore, the nucleotide sequences that had been derived were compared with other related sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) alignment algorithm (http://www.ncbi.nlm.nih.gov/BLAST/).

Capsule gene clusters sequence analysis

The PCR products of capsule gene cluster for the 15 strains of *Lactococcus garvieae* isolate from various places were sequenced directly with the same primers used for PCR assay. Afterward, a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) was used for the sequencing of each PCR product. To form a continuous sequence of the DNA that had been amplified, forward and reverse nucleic acid sequence data for the capsule gene cluster region were utilized. Using some available sequences in NCBI (National Center for Biotechnology Information) and BLAST, the continuous sequences were compared with each other. In addition, the MEGA 6 program (Research Center for Genomics and Bioinformatics, Japan), via FASTA algorithms, was used to both perform multiple-sequence alignment analysis and construct a phylogenetic tree. The construction of the trees was done based on the maximum likelihood (ML) method (Sneath and Sokal, 1973); also, MEGA version 6 (Research Center for Genomics and Bioinformatics, Japan) was used to estimate the evolutionary distances (Tamura *et al*., 2007).

Results

Totally, 22 strains of *Lactococcus garvieae* isolated from various places were examined for the existence of virulence genes. All strains of *L. garvieae* entailed the *adhPsaA*, *LPxTG-3*, *hly3*, *NADH oxidase*, *hly2*, *pgm* (Fig. 2) *sod*, *eno*, *adhCI*, *hly1* (Fig. 3) *adhPav* and *CGC* genes *adhCII*. Also, *adhCII* gene was present in all strains except one of the isolates originated from mugger crocodile (Fig. 3) (Table 2). *LPxTG-2* was present in one of the isolates of mugger crocodile and *adhesion* genes were not identifiable in all the isolates (Table 2). The result of electrophoresis showed that 285 bp fragments amplified from all strains of *L. garvieae* were serotype I.

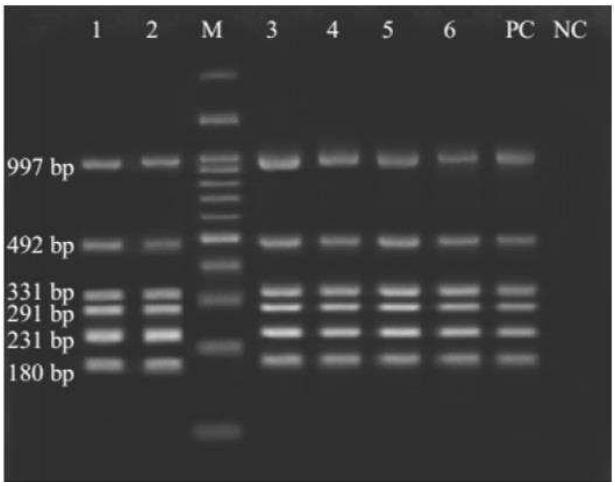

Fig. 2: *Adhesin PsaA*, *LPxTG-3*, *Hemolysin 3*, *NADH oxidase*, *Hemolysin 2* and *Phosphoglucomutase* genes detection with multiplex PCR technique. M: DNA ladder (100 bp), Lanes 1 to 6: Positive samples of *L. garvieae* compared to positive control (PC) and negative control (NC)

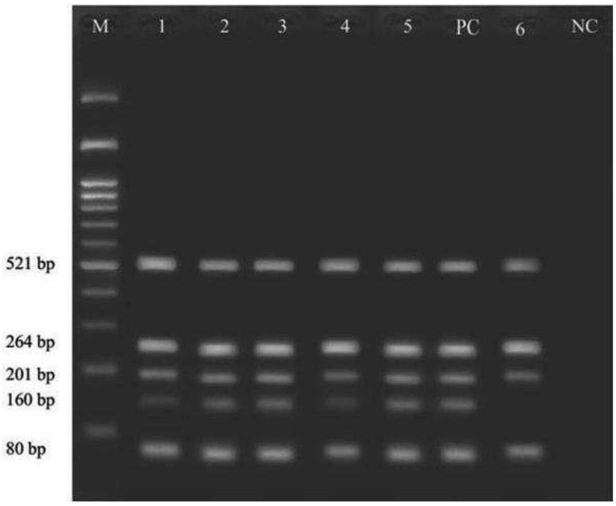

Fig. 3: *Superoxide dismutase*, *Adhesin cluster 2*, *Enolase*, *Adhesin cluster 1* and *Hemolysin 1* genes detection with multiplex PCR technique. M: DNA ladder (100 bp), Lanes 1 to 5 rainbow trout and Lane 6 mugger crocodile positive samples of *L. garvieae* compared to positive control (PC), and negative control (NC)

The results obtained from the sequencing of PCR products confirmed that the correct products were in fact amplified by the primers, with the amplified fragment precisely corresponding to the database sequence of the gene that had been targeted.

The staining of the capsule, and simplex PCR results, indicated that the capsule was detectable in all the strains.

All fish strains and one isolate obtained from the mugger crocodile shared 100% capsule gene cluster sequences similarity together. However, another strain of mugger crocodile showed different capsule gene sequence. Based on this molecular marker, Iranian isolates showed 99.94% to 99.93% homology ranges with the other GenBank accession numbers of the *Lactococcus garvieae.* The partial sequence analysis of the Iranian fish isolates showed 99.94% similarity to the accession number AP017373 (*Lactococcus garvieae*, Japan) and 99.93% similarity to the accession numbers AP009332 and AP009333.

The phylogenetic tree was constructed using the maximum composite likelihood method (Fig. 4). The sequences of capsule gene cluster in *L. garvieae* strains from different geographic origins (Table 2) were phylogenetically assigned in three major genetic groups (A, B and C). All fish isolates grouped into a cluster together with one isolate obtained from the mugger crocodile. While another strain of mugger crocodile belonged to phylogenetic group B, molecular phylogenetic evidence demonstrated that groups A and B were sister groups. The sequencing result of the amplified products obtained in this study has been registered in GenBank under the accession number

Table 2: Presence of virulence genes in *Lactococcus garvieae* isolates. *Hemolysins* 1, 2, and 3, *NADH oxidase*, *phosphoglucomutase*, *adhesin Pav*, *superoxide dismutase*, *adhesin PsaA*, *enolase*, and *adhesin clusters* 1 were found in all isolates

Isolate	Host	Virulence genes												
		Hly 1, 2, 3	*pgm*	*eno*	*adhCI*	*adhCII*	*sod*	*LPxTG-2*	*LPxTG-3*	*NADH oxidase*	*aPav*	*aPsaA*	*adhesion*	*CGC*
Tehran 1	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Tehran 2	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Mazandaran 1	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Mazandaran 2	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Lorestan	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Kohgiluyeh and Boyer-Ahmad	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Kohgiluyeh and Boyer-Ahmad	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Kohgiluyeh and Boyer-Ahmad	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Kohgiluyeh and Boyer-Ahmad	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Kohgiluyeh and Boyer-Ahmad	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Kohgiluyeh and Boyer-Ahmad	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Fars	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Fars	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Fars	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Fars	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Fars	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Chaharmahal and Bakhtiari	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Chaharmahal and Bakhtiari	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Chaharmahal and Bakhtiari	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Chaharmahal and Bakhtiari	Rainbow trout	+	+	+	+	+	+	−	+	+	+	+	−	+
Sistan and Balouchestan 1	Mugger crocodile	+	+	+	+	−	+	−	+	+	+	+	−	+
Sistan and Balouchestan 1	Mugger crocodile	+	+	+	+	+	+	+	+	+	+	+	−	+

Hly: *Hemolysins*, *pgm*: *Phosphoglucomutase*, *eno*: *Enolase*, *adhCI*: *Adhesin clusters 1*, *adhCII*: *Adhesin clusters 2*, *sod*: *Superoxide dismutase*, *aPav*: *Adhesin Pav*, *aPsaA*: *Adhesin PsaA*, and *CGC*: *Capsule gene cluster*. + Indicates gene is present, and − indicates gene is absent

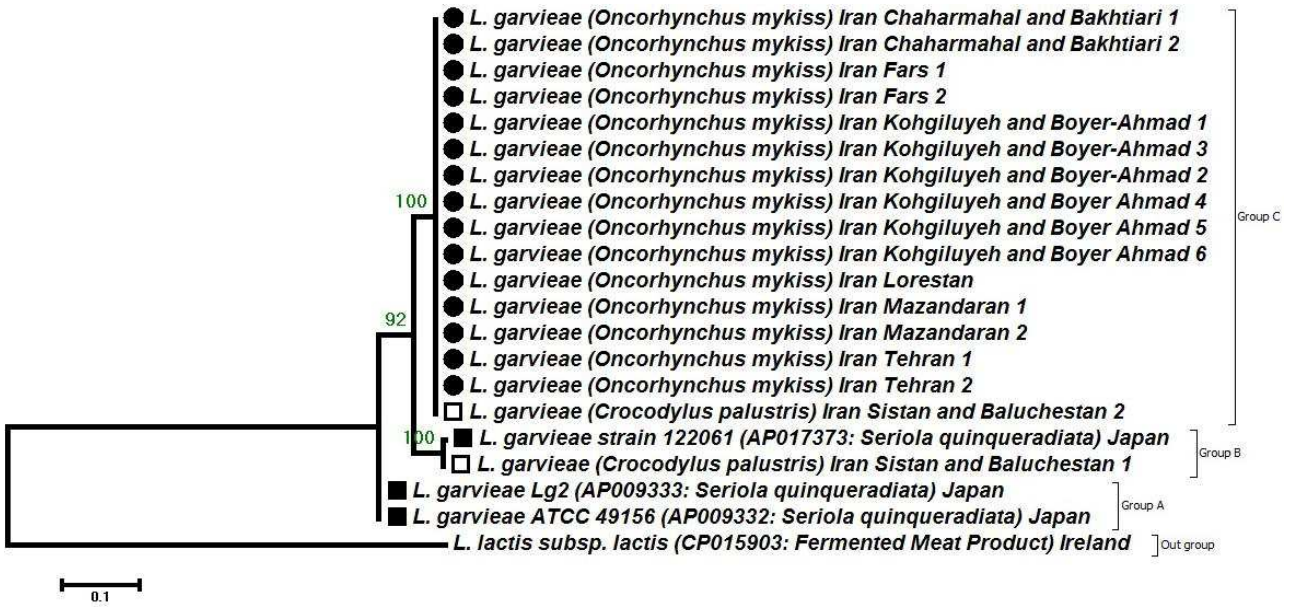

Fig. 4: Molecular phylogenetic analysis of the Iranian isolate capsule gene cluster of *Lactococcus garvieae* obtained from *Oncorhynchus mykiss* (●) and *Crocodylus palustris* (□) in the present study and other related sequences existing in the GenBank (■) based on the partial capsule gene cluster sequences. The molecular evolutionary analysis was conducted using maximum likelihood (ML) method in the MEGA 6.0 program. Numerals above the branches indicate bootstrap values (%) from 1,000 replicates. The scale bar indicates the proportion of sites changing along each branch

MH028744 to MH028754, MH042028 to MH042042, MH443411, MH463453, MH463454, MH059478, and MH059479.

Discussion

Recently, a simplex PCR has been developed to detect the capsule gene cluster of *L. garvieae*, in the current study the specificity of the mentioned assay was also verified again using Iranian strains of *L. garvieae*. In all cases, the visibility of the PCR product bands on gel electrophoresis was confirmed, and the generated positive findings were similar to the results reported in Miyauchi *et al.*'s (2012) and Raissy *et al.*'s (2016) studies. Miyauchi *et al*. (2012) found that the capsule gene cluster was detectable only in pathogenic *L. garvieae* strains as a genomic island. However, and considering the non-capsulated *L. garvieae* strains, ATCC 49156 and Lgper were found to be virulent to rainbow trout with a mortality rate of 98% and 89%, respectively (Türe *et al*., 2014). Therefore, it is likely that this virulence gene alone may not be involved in implicating the causative agency of *L. garvieae* to rainbow trout; other pathogens may also play a role in this regard.

Based on the phylogenetic sequences of the capsule gene cluster, strains from distinct hosts were assigned in two major genetic groups (A and B). All fish isolates grouped into a cluster together with one isolate obtained from mugger crocodile. These genetic diversity results probably show an adaptation of different strains of this pathogen to the host species.

In the current study, in order to detect and characterize 11 virulence genes in *L. garvieae*, two multiplex PCR assays were developed. The two multiplex PCR techniques proved to be useful and functional in simultaneously detecting virulence genes in *L. garvieae* from different sources.

It is stated that *hly1*, *hly2*, *hly3*, *NADH oxidase*, *sod*, *pgm*, *adhPav*, *adhPsaA*, *eno*, *LPxTG*, *adhCI*, *adhCII*, and *adh*, as well as the capsule gene cluster, have a role in the virulence of *L. garvieae* (Miyauchi *et al*., 2012). Nonetheless, in order to refer to a strain as a pathogen, it is not enough that the given strain possesses only a single or a few virulence genes. In fact, for a strain to be able to be considered a causative agent in a specific host species, it must have obtained the appropriate virulence gene combination. Coutte *et al*. (2003) explained that adhesions are among the most significant virulent genes that facilitate bacterial adhesion to other bacteria or to the surface of the host cells. The isolates of *L. garvieae*, belonging to human and fish, were found to hold specific adhesion genes (Miyauchi *et al*., 2012). In the current study *adhPav*, *adhCI*, and *adhPsaA* genes were identifiable in all the strains; this finding was in agreement with those reported by Miyauchi *et al*. (2012) and Ture and Altinok (2016). *adhCII* gene was present in all the strains except in one of the isolates of mugger crocodile and *Adhesin* gene was not detected in all strains. However, Miyauchi *et al*. (2012) and Ture and Altinok (2016) revealed the presence of this gene in some strains in Japan, Turkey, France, Italy, and Spain.

LPxTG proteins play essential roles in bacterial virulence which makes covalent bonds in conjunction with the peptidoglycan detached from various Gram-positive bacteria (Miyauchi *et al*., 2012). In the present study, an effort was made to explore the existence of two types of *LPxTG* genes. In all of the isolates, the presence

of *LPxTG-3* gene was confirmed, similar to findings from Ture and Altinok's (2016) study. However, *LPxTG-2* gene was found in one isolate.

Superoxide dismutases (SODs) facilitate the process of the dismutation of superoxide radicals into oxygen or hydrogen peroxide. Some bacteria yield SODs during the course of infection to protect themselves from being killed (Vanaporn *et al*., 2011). In the present study, *sod* virulence genes were spotted in all Iranian isolates and this finding was in line with what Miyauchi *et al*. (2012) and Ture and Altinok (2016) had found in their studies.

Other virulence genes are *Enolase* (*eno*), *phosphoglucomutase* (*PG*), and *NADH oxidase*. Enolase and PG are considered to be among the metabolic enzymes, yet they are also thought to be moonlighting proteins which have a role in the virulence of some bacteria. However, even though *eno* and *PG* are the immunogenic proteins found present in the cell extracts of *L. garvieae* (Shin *et al*., 2009). In the current study, the presence of these three genes was established in all isolates, similar to the findings of Miyauchi *et al*. (2012) and Ture and Altinok (2016). Therefore, the virulence of a bacterium that lacks capsule gene cluster gene may come from potential virulence factors coded by *SOD*, *NADH oxidase*, *PG*, and *eno* (Morita *et al*., 2011).

Most bacteria and fungi release hemolysin (Stipcevic *et al*., 2005). Miyauchi *et al*. (2012) also identified hemolysin genes in *L. garvieae* isolates. In this study, the existence of three distinct *hemolysin* genes (*hly1*, *hly2*, *hly3*) was investigated, and it was found that all the isolates contained all the hemolysin genes; a finding which was in line with those reported by Miyauchi *et al*. (2012) and Ture and Altinok (2016).

In this research, all the strains of *L. garvieae* were categorized as serotype I following the application of a specific PCR method. Recently, this molecular technique was developed by Ohbayashi *et al*. (2017). Based on these results, the lactococcosis in Iran is suggested to have been caused by serotype I, the only recognized serotype in this study.

In conclusion, further studies are recommended to investigate the expression of virulence genes in the *in vivo* and *in vitro* conditions and their genetic diversities in *L. garvieae* and evaluate their pathogenicity to rainbow trout.

Acknowledgement

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Conflict of interest

The authors declare that they have no conflict of interest.

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