Archive of SID

ORIGINAL ARTICLE

Veterinary Research Forum. 2019; 10 (2) 101 - 107
doi: 10.30466/vrf.2018.89527.2164
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Determination of virulence associated immunogenic proteins in some of *Lactococcus garvieae* strains

Meryem Cansu Yesiltas, Ilhan Altinok*, Rafet Cagri Ozturk

Department of Fisheries Technology Engineering, Surmene Faculty of Marine Sciences, Karadeniz Technical University, Trabzon, Turkey.

Article Info

Article history:

Received: 10 July 2018
Accepted: 25 September 2018
Available online: 15 June 2019

Key words:

Immunogenic protein
Lactococcosis
Rainbow trout
Western blot
Yellowtail

Abstract

Lactococcosis disease incident caused by *Lactococcus garvieae* has been increased with increasing aquaculture productions and outbreaks of the disease have become a threat on farmed species. To prevent lactococcosis, inactivated vaccine has been used, however, it only provides protection when given by injection. Other than inactivated vaccine, various vaccines such as subunit vaccines can be developed. In the present study, total protein profile of 43 strains of *L. garvieae* isolated from fish, milk and cheese by SDS-PAGE and virulence associated immunogenic proteins of *L. garvieae* strains using western blot with hyper-immune rabbit sera were determined. After analyzing whole-cell lysate protein of *L. garvieae* strains with SDS-PAGE, protein bands were ranged between 8.00 and 140.00 kDA. Among strains, variable protein bands were ranged between 17.00 and 48.00 kDa with some variability in the staining intensity of the protein bands and formed in 6 clusters. The immunogenic protein bands were ranged between 25.00 - 75.00 kDa. Only a variable and highly immunogenic protein band was observed between 40.00 and 45.00 kDa. Most of the strain including Lgper had 44.00 kDa immunogenic protein while nonvirulent ATCC strain had 42.50 kDA immunogenic protein. Predominant immuno-reactive proteins encoded by genes can be used as a subunit vaccine.

© 2019 Urmia University. All rights reserved.

تعیین حدت مرتبط با پروتئین های ایمنوژنیک در برخی از سویه های *لاکتوکوکوس گارویه*

چکیده

شیوع بیماری لاکتوکوکوز با عامل مولد *لاکتوکوکوس گارویه* با افزایش تولیدات آبزی پروری رو به فزونی گذاشته است و وقوع بیماری در گونه های پرورشی به یک تهدید تبدیل شده است. به منظور جلوگیری از لاکتوکوکوز، واکسن غیر فعال مورد استفاده قرار می گیرد. اما این نوع واکسن صرفاً از طریق تزریقی ایمنی ایجاد می کند. واکسن های متفاوتی به غیر از واکسن غیر فعال نظیر واکسن های تحت واحد را می توان تهیه کرد. در مطالعه حاضر، پروفایل پروتئین تام ۴۳ گونه از *لاکتوکوکوس گارویه* جدا شده از ماهی، شیر و پنیر با استفاده از SDS-PAGE و پروتئین های ایمنوژنیک مرتبط با حدت گونه های *لاکتوکوکوس گارویه* از طریق وسترن بلات همراه با سرم های خرگوشی هیپرایمیون تعیین شدند. بعد ازآنالیز پروتئین لیزات، سلول گونه های *لاکتوکوکوس گارویه* با SDS-PAGE، باند های پروتئینی بین ۸/۰۰ تا ۱۴۰/۰۰ کیلو دالتون و متغیر پروتئینی بین ۱۷/۰۰ تا ۴۸/۰۰ کیلو دالتون (با تنوع در شدت رنگ پذیری باندها) قرار گرفتند و شش گروه تشکیل دادند. باند های پروتئینی ایمنوژنیک بین ۲۵ تا ۷۵ کیلو دالتون قرار داشتند. صرفاً یک پروتئین متغیر و بسیار ایمنوژنیک بین ۴/۰۰ تا ۴۵/۰۰ کیلو دالتون قرار گرفت. بیشتر سویه ها ی واجد Lgper دارای پروتئین ایمنوژنیک ۴۴/۰۰ کیلو دالتونی بودند، درحالیکه سویه غیر حاد ATCC دارای پروتئین ایمنوژنیک ۴۲/۵۰ کیلو دالتونی بود. پروتئین های ایمنو راکتیو غالب که با ژن ها کدبندی شده اند را می توان به عنوان تحت واحد واکسن مورد استفاده قرار داد.

واژه های کلیدی: پروتئین ایمنوژنیک، دم زرد، قزل آلای رنگین کمان، لاکتوکوکوز، وسترن بلات

***Correspondence:**

Ilhan Altinok. PhD
Department of Fisheries Technology Engineering, Faculty of Marine Science, Karadeniz Technical University, Trabzon, Turkey
E-mail: ialtinok@ktu.edu.tr

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License which allows users to read, copy, distribute and make derivative works for non-commercial purposes from the material, as long as the author of the original work is cited properly.

www.SID.ir

Archive of SID

Introduction

Lactococcus garvieae, gram-positive cocci bacteria, is the causative agent of the lactococcosis, known as an infectious systemic disease.[1] Lactococcosis disease affects both freshwater and saltwater fish[2-4] including yellowtail (*Seriola quinqueradiata*),[5] rainbow trout (*Oncorhynchus mykiss*)[6] and black rockfish (*Sebastes schlegelii*).[7] *Lactococcus garvieae* is also considered as a zoonotic agent capable of causing disease in different animal species and humans.[8] In Turkey, lactococcosis was first reported in Aegean Region[9] and it started to spread across the country. The pathogen causes serious losses in cultured fish especially in farmed rainbow trout.[1]

Conventionally, diagnosis of the lactococcosis disease is achieved by agar cultivation and then biochemical and phenotypic properties of the bacteria.[10,11] Molecular techniques can also be used to identify *L. garvieae* directly from host tissue without culturing bacteria.[12] Serological characteristics of *L. garvieae* have been widely studied to characterize the strains. *L. garvieae* strains can be classified as: Capsulated (KG^-) and non-capsulated (KG^+) strains.[13] KG^- strain is agglutinated by anti KG^-serum. Meanwhile, KG^+ strain is agglutinated by both anti KG^- and anti KG^+ sera.[14] Serotype KG^- are hydrophilic, resistant to phagocytic ingestion and more virulent compared to serotype KG^+.[15] On the other hand, presence of capsule gene is not directly related to virulence of *L. garvieae*. Ture and Altinok[16] found that all the Turkish *L. garvieae* isolates lacked capsule gene, however, they were virulent.

Sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is fairly fast, easy and reproducible technique for whole cell protein pattern analysis.[17] The SDS-PAGE protein profile analyses can be used to identify inner membrane proteins,[18] outer membrane proteins[19] and quantification of allergen proteins.[20] For antigen expression or vaccine efficiency, antigenic compound of the bacteria should be recognized by fish. Limited research has been conducted on the whole cell protein and antigenic variety of *L. garvieae*.[15,21] The aims of the present study were to determine total protein profile of various *L. garvieae* strains by SDS-PAGE and to determine virulence associated immunogenic proteins of *L. garvieae* strains using western blot with hyper-immune rabbit sera.

Materials and Methods

Bacterial strains. *Lactococcus garvieae* strains (n = 43) were obtained from different fish farms located in Aegean and Black Sea regions of Turkey and also from Spain, Italy, France and Japan (Table 1). Genetic and biochemical properties of the *L. garvieae* strains were determined prior to this study and none of the strains had capsule protein gene.[16] The ATCC49156 strain (avirulent) was used as a negative control and Lgper strain (virulent) was used as a positive control. Prior to the experiment, rainbow trout (78.00 ± 8.50 g) were challenged with both strains and Lgper strain caused 74.00% mortality while ATCC 49156 strain did not cause any mortality. All the bacteria were subcultured on trypticase soy agar (TSA) to ensure purity of the colonies (Table 1).

Rabbits. Animals used in antibody generation were six months old male New Zealand rabbits (n = 3) weighing 2.50 - 3.00 kg each. The rabbits were obtained from Firat University and quarantined for 10 days prior to the study. Throughout the study, animals were held in Karadeniz Technical University, Faculty of Medicine. Animal studies were carried out with the guidelines of the Institutional Animal Care and Use Committee at the Karadeniz Technical University (approval #11/2013).

Preparation of antigen and antibody generation. Lgper strain was used to produce hyper-immune rabbit sera. Bacteria were incubated overnight at 29.00 ˚C on tryptic soy agar (TSA). Then single colony was subcultured in brain heart infusion broth (BHIB) at 29 ˚C for 5 hr. The bacteria concentration was adjusted to an optical density of 0.60 (OD_{630} 0.60 × 10^9 CFU mL^{-1}) using Shimadzu UV-2550 (Tokyo, Japan) spectrophotometer. Bacteria were inactivated by 0.70% (v/v) formaldehyde as described by Huang *et al*. with slight modifications.[22] After inactivation procedure, samples were centrifuged at 4,000 *g* for 5 min and then bacterial pellet was re-suspended in phosphate-buffered saline (Sigma Aldrich, Taufkirchen, Germany) at final concentration of 1.40 × 10^9 CFU mL^{-1} and stored at 4 ˚C. For injection, 3.00 mL of Freund's Complete Adjuvant (Sigma-Aldrich) was mixed with equal amount of formalin-inactivated bacterial solution. Each rabbit was injected subcutaneously with 1.00 mL of the solution three times with a two-week interval. Blood samples were collected from marginal ear vein of the rabbits after two weeks post-injection and serum was obtained by centrifugation at 4000 *g* for 15 min at 4˚C and then stored at – 80 ˚C.

Extraction of bacterial proteins and protein profile by SDS-PAGE. Bacteria were cultured in BHIB and incubated at 29 ˚C until bacterial optical density was reached to 2 (OD_{630}). Bacteria culture was washed twice with PBS and optical density adjusted to 2 (OD_{630}) for each strain. Bacteria were sonicated three times (Bandelin Electronic, Berlin, Germany) at 20 Watt on ice for 2 min and then bacterial cells were disrupted by boiling for 5 min. The cell debris were removed by centrifugation at 10.000 *g* for 10 min at 4˚C. Protein concentrations were measured using bicinchoninic acid (BCA) protein assay kit (BioVision Inc., Milpitas, USA) according to the manufacturer's instructions. Proteins were separated on 4.00 - 12.00% SDS-PAGE gels by loading 15.00 µL samples per lane. The electrophoresis was performed at 125 V, 41 mA for 65 min. Protein fragments were visualized by silver staining according to Winkelstroter *et al*.[23]

www.SID.ir

Archive of SID

Table 1. *Lactococcus garvieae* strains used in whole-cell protein profile and western blot, (T = Turkey).

No	Strain	Host	Origin	Isolated in
1	**1684**	Rainbow trout	Spain	1997
2	**2398**	Rainbow trout	France	1998
3	**M1**	Rainbow trout	Muğla (T)	2002
4	***L. lactis***	Cow milk	Isparta, (T)	2005
5	**AA**	Rainbow trout	Rize (T)	2008
6	**Pap225-1**	Rainbow trout	Artvin (T)	2008
7	**K9**	Rainbow trout	Gümüşhane (T)	2010
8	**8053 C/02**	Rainbow trout	Spain	2000
9	**164 A/03**	Rainbow trout	Spain	2000
10	**04/8782 FTPI**	Rainbow trout	Spain	2001
11	**04/8782 (532)**	Rainbow trout	Spain	2001
12	**04/8782 (498)**	Rainbow trout	Spain	2001
13	**04/8782 ITP109**	Rainbow trout	Spain	2002
14	**8740/03**	Rainbow trout	Spain	2003
15	**1073/03C**	Rainbow trout	Spain	2000
16	**M300**	Goat cheese	Italy	2001
17	**A-58**	Rainbow trout	Italy	2002
18	**G-27**	Cow milk	Italy	2003
19	**AF-14**	Rainbow trout	Rize (T)	2009
20	**PP6O**	Rainbow trout	Italy	2004
21	**M2**	Rainbow trout	Muğla (T)	2002
22	**M3**	Rainbow trout	Muğla (T)	2003
23	**M5**	Rainbow trout	Muğla (T)	2009
24	**M4**	Rainbow trout	Muğla (T)	2011
25	**216-6**	Rainbow trout	Rize (T)	2007
26	**AA-37**	Rainbow trout	Rize (T)	2008
27	**512-8**	Rainbow trout	Rize (T)	2008
28	**OME**	Rainbow trout	Rize (T)	2008
29	**511-15**	Rainbow trout	Rize (T)	2008
30	**Isfk**	Rainbow trout	Rize (T)	2009
31	**Sider-17**	Rainbow trout	Rize (T)	2008
32	**399-18**	Rainbow trout	Artvin (T)	2008
33	**BB**	Rainbow trout	Artvin (T)	2008
34	**636-16**	Rainbow trout	Gümüşhane (T)	2008
35	**671-14**	Rainbow trout	Gümüşhane (T)	2008
36	**670-20**	Rainbow trout	Gümüşhane (T)	2008
37	**673-5**	Rainbow trout	Gümüşhane (T)	2008
38	**235-16**	Rainbow trout	İzmir (T)	2009
39	**R-817**	Rainbow trout	Trabzon (T)	2014
40	**Ser114**	Rainbow trout	Trabzon (T)	2009
41	**Trb**	Rainbow trout	Trabzon (T)	2011
42	**A30**	Rainbow trout	Rize (T)	2009
43	**ATCC 49156**	Yellowtail	Japan	1974
44	**Lgper**	Rainbow trout	Ordu (T)	2011

Western blotting. Proteins were again resolved by 5.00 - 12.00% SDS-PAGE gels by loading 15.00 µL samples per lane. The electrophoresis was performed at 80.00 V (500 mA, 150 W) for 10 min and at 110 V for 90 min. Proteins were transferred to 0.45 µm pore size nitrocellulose membranes (Bio-Rad, Hercules, USA) at 35 V (500 mA, 150 W) for 1 hr and blocked with 5.00% non-fat milk in tris-buffered saline with Tween 20 (TBST; 20 mM Tris-HCl (Sigma Aldrich), 150 mM NaCl (Sigma Aldrich) , 0.05%, 0.10% Tween 20 (Sigma Aldrich) for 1 hr on vertical shaker. The membranes were incubated with hyper-immune rabbit sera (primary antibodies) at 1:1000 dilutions for 1 hr at room temperature and washed three times with TBST with 10 min intervals. Subsequently incubated with mouse anti-rabbit IgG-HRP secondary antibody (1:10000; Santa Cruz Biotechnology Inc., Dallas, USA) at room temperature for 1 hr and washed three times with TBST. Protein bands were visualized using ChemiDoc imaging system (Bio-Rad, Hercules, USA) with ECL Prime kit (Merck Millipore, Burlington, USA). For negative control nonimmune rabbit sera were used as primary antibodies. In order to determine specification of antibody or sera obtained from rabbits after exposing to *L. garvieae* Lgper strains, western blotting was performed with *Enterobacter cloacae*, *Aeromonas hydrophila*,

www.SID.ir

Archive of SID

Pseudomonas fluorescens, *Streptococcus iniae*, *Yesinia ruckeri*, *Vibrio fluvialis*, *P. fluorescens*, *P. putida*, *Listonella anguillarum*, *Vibrio parahaemolyticus* and *P. luteola* whole cell proteins.

Statistical Analyses. Band patterns were analyzed with Bionumerics GelCompar II (Applied Maths, Brussels, Belgium). The similarities between band patterns of different isolates were calculated using the Dice similarity coefficient with a 2.50% tolerance and 1.00% optimization. The dendrogram was constructed using cluster analysis unweighted pair-group method with arithmetic mean (UPGMA). Discriminatory indices (DIs) of the typing methods were calculated based on the formula developed by Hunter and Gaston.[24]

Results

After analyzing whole-cell lysate protein of *L. garvieae* strains with SDS-PAGE, protein bands were ranged between 8.00 and 140.00 kDA. Among strains, number of fragments were ranged between 7 - 17 and variable protein bands were ranged between 17.00 and 48.00 kDa with some variability in the staining intensity of the protein bands and formed in six clusters (Fig. 1).

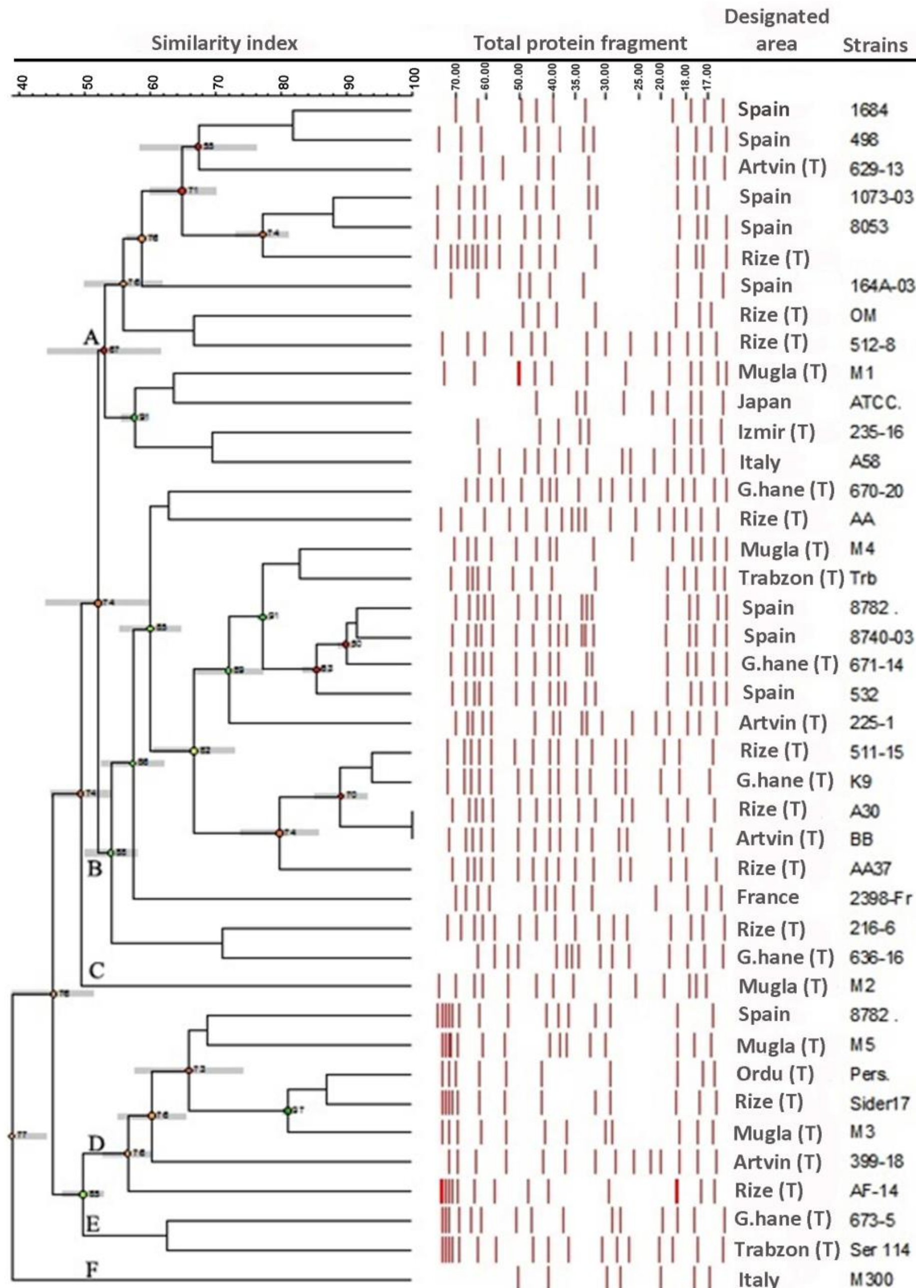

Fig. 1. Dendrogram obtained from SDS-PAGE whole-cell protein profiles of *Lactococcus garvieae*.

www.SID.ir

Archive of SID

According to the Dice similarity coefficient, similarity rates among strains were found to be low (≤ 40.00 %), (Fig. 2). Cluster A was composed of two sub-clusters; A1 and A2. Spain (n = 5) and Eastern Black Sea region of Turkey (n = 4) strains formed sub-cluster A1 with 55.80 ± 5.90% similarity, meanwhile Mugla (M1), Izmir (235-16), ATCC 49156 and Italy (A58) strains formed sub-cluster A2 with 57.80 ± 1.80% similarity. Similarity between sub-cluster A1 and A2 was determined as 53.00 ± 8.00%. Cluster B consisted of 17 strains which were grouped in three sub-clusters with similarity of 59.90 ± 3.90%. Similarity between Cluster A and B was determined as 52.00 ± 7.90%. Cluster C consisted of only France (2398) strain and its similarity with Cluster A and B was 49.40 ± 4.50%. Spain (8782), Mugla (M3, M5), Ordu (Lgper), Rize (Sider17, Af-14) and Artvin (399, 18) grouped in cluster D with 56.60 ± 3.80% similarity. Gumushane (676-5) and Trabzon (Ser114) grouped in cluster E. Italy (M300) strain isolated from goat cheese formed cluster F with 38.90 ± 5.30% similarity with other clusters (Fig. 1).

Molecular weights (MV) of the immunogenic proteins were ranged between 25.00 - 75.00 kDa. All of the *L. garvieae* strains had identical western blot patterns except for R-817 and Ser114 strains isolated from Trabzon, and only a single variable and highly immunogenic protein band were observed between 40.00 and 45.00 kDa. Most of the strain including Lgper had 44.00 kDa immunogenic protein while nonvirulent ATCC strain had 42.50 kDA immunogenic protein.

Lactococcus lactis and 04/8782 ITP109 Spain strain had similar pattern that was completely different from the other *L. garvieae* strains (Fig. 2).

Specificity of the Lgper antibody was tested against 11 different bacteria. While *Enterobacter cloacae* and *A. hydrophila* had weak antigenic band similar to Lgper, the other bacteria did not have antigenic band indicating the specificity of Lgper serum obtained from rabbits (Fig. 3).

Fig. 3. Reaction of *Lactococcus garvieae* antibody with other bacteria species M: Marker (-): ATCC 49156, (+): Lgper, 1: *Enterobacter cloacae*, 2: *Aeromonas hydrophila*, 3: *Pseudomonas fluorescens* 4: *Streptococcus iniae*, 5: *Yesinia ruckeri*, 6: *Vibrio fluvialis*, 7: *P. fluorescens*, 8: *P. putida*, 9: *Listonella anguillarum* 10: *V. parahaemolyticus*, 11: *P. luteola.*

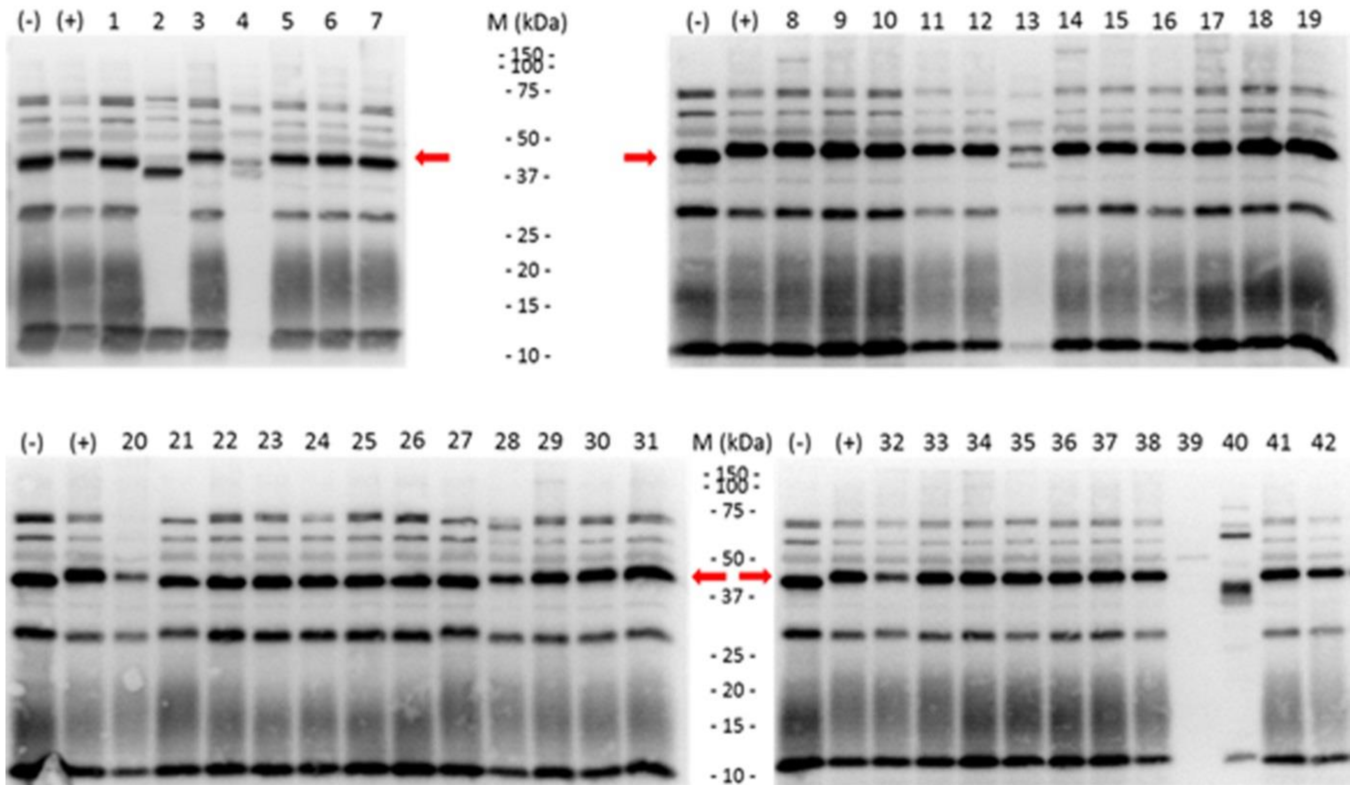

Fig. 2. Western blot images of *Lactococcus garvieae* and *L. lactis* strains. (-) and (+) indicate ATCC 49156 and Lgper strain, respectively. Please check the Table 1 for the strains.

www.SID.ir

Archive of SID

Discussion

The SDS-PAGE can be used to identify species and subspecies of bacteria.[25,26] *Lactococcus garvieae* strains used in the present study were confirmed by PCR and biochemical tests in previous studies.[4,16] Although all the strains were confirmed as *L. garvieae*, they had different whole protein profile based on analysis by SDS-PAGE. Six clusters were formed depending on strains isolated. *Lactococcus garvieae* M300 isolated from goat cheese in Italy had thoroughly different whole cell protein profile than the other *L. garvieae* strains; however, its immuno-genic protein profile was similar to that of the other strains. Furthermore, immunogenic properties of antigenic sera obtained from rabbit was specific to *L. garvieae*, because, it did not form cross reactivity to other bacterial species.

Lactococcosis is a systemic fish disease affecting many species. The disease agent was first isolated in the Aegean Region and then it was spread to every region where rainbow trout was cultured in a short time and it caused high mortalities.[1] Antibiotics are commonly used to treat the lactococcosis disease and most of the strains gain resistance to them.[27,28] Furthermore, to prevent lactococcosis, inactivated vaccine has been used, however, it usually only provides protection when applied by injection. Immersion or oral application does not provide remarkable protection.

In order to help prevent environmental pollution and reduce antibiotic usage, development of an efficient vaccine such as subunit vaccine against lactococcosis is vital. In light of the results obtained from this study, highly immunogenic protein band was observed between 40.00 and 45.00 kDa. Most of the strain including Lgper had 44.00 kDa protein while nonvirulent ATCC strain had 42.50 kDA protein. Predominant immunogenic proteins encoded by genes can be used as a subunit vaccine.

The GAPDH, a cytosolic glycolytic protein, is a path of the outer membrane proteins. The GAPDH is responsible for pathogenesis or it is a virulence associated immune-modulatory protein.[29] The GAPDH gene encodes 44.00 kDa protein and it has immunoprotective properties.[30] In the present study, antigenic protein band was observed between 37.00 and 50.00 kDa. Therefore, this protein could be responsible for the virulence of *L. garvieae* and it could be used as a subunit vaccine.

Acknowledgments

The authors thank Dr. Zihni Acar Yazici, Dr. Ersan Kalay, Dr. Celal Kurtuluş Buruk and Mr. Adem Yildirim for technical assistance and veterinarian Sait Al for taking care of rabbits.

Conflict of interest

The authors declare that there is no conflicts of interest regarding the publication of this article.

References

1. Ozturk RC, Altinok I. Bacterial and viral fish diseases in Turkey. Turk J Fish Aquat Sci 2014; 14: 275-297.
2. Austin B, Austin DA. Bacterial fish pathogens; diseases of farmed and wild fish. 5th ed. London, UK: Springer2012; 17-58.
3. Plumb JA, Hanson LA. Health maintenance of cultured fishes: Principal microbial diseases. 3rd ed. Iowa, USA: Wiley-Blackwell 2011; 483.
4. Ture M, Altinok I, Capkin E. Comparison of pulsed-field gel electrophoresis and enterobacterial repetitive intergenic consensus PCR and biochemical tests to characterize *Lactococcus garvieae*. J Fish Dis 2015; 38(1): 37-47.
5. Park KH, Matsuoka S, Nakai T, et al. A virulent bacteriophage of *Lactococcus garvieae* (formerly *Enterococcus seriolicida*) isolated from yellowtail *Seriola quinqueradiata*. Dis Aquat Organ 1997; 29: 145-149.
6. Chang PH, Lin CW, Lee YC. *Lactococcus garvieae* infection of cultured rainbow trout, *Oncorhynchus mykiss*, in Taiwan and associated biophysical characteristics and histopathology. B Eur Assoc Fish Pat 2002; 22(5): 319-327.
7. Kang SH, Shin GW, Shin YS, et al. Experimental evaluation of pathogenicity of *Lactococcus garvieae* in black rockfish (*Sebastes schlegeli*). J Vet Sci 2004; 5(4): 387-390.
8. Ghasemi SM, Bouzari M, Yoon BH, et al. Comparative genomic analysis of *Lactococcus garvieae* phage WP-2, a new member of Picovirinae subfamily of Podoviridae. Gene 2014; 551(12): 222-229.
9. Diler O, Altun S, Adiloglu AK, et al. First occurrence of Streptococcosis affecting farmed rainbow trout (*Oncorhynchus mykiss*) in Turkey. B Eur Assoc Fish Pat 2002; 22(1): 21-26.
10. Eldar A, Goria M, Ghittino C, et al. Biodiversity of *Lactococcus garvieae* strains isolated from fish in Europe, Asia, and Australia. Appl Environ Microbiol 1999; 65(3): 1005-1008.
11. Ravelo C, Magarinos B, Herrero MC, et al. Use of adjuvanted vaccines to lengthen the protection against lactococcosis in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 2006; 251(2-4): 153-158.
12. Altinok I. Multiplex PCR assay for detection of four major bacterial pathogens causing rainbow trout disease. Dis Aquat Organ 2011; 93(3): 199-206.
13. Soltani M, Mohamadian S, Ebrahimzahe-Mousavi HA, et al. Shirazi thyme (*Zataria multiflora*) essential oil suppresses the expression of the epsD capsule gene in

www.SID.ir

Archive of SID

Lactococcus garvieae, the cause of lactococcosis in farmed fish. Aquaculture 2014; 433: 143-147.

14. Kawanishi M, Yoshida T, Yagashiro S, et al. Differences between *Lactococcus garvieae* isolated from the genus Seriola in Japan and those isolated from other animals (trout, terrestrial animals from Europe) with regard to pathogenicity, phage susceptibility and genetic characterization. J Appl Microbiol 2006; 101(2): 496-504.
15. Ooyama T, Hirokawa Y, Minami T, et al. Cell-surface properties of *Lactococcus garvieae* strains and their immunogenicity in the yellowtail *Seriola quinqueradiata*. Dis Aquat Organ 2002; 51(3): 169-177.
16. Ture M, Altinok I. Detection of putative virulence genes of *Lactococcus garvieae*. Dis Aquat Organ 2016; 119(1): 59-66.
17. Sanchez L, Martinez P, Vinas A, et al. Analysis of the structure and variability of nucleolar organizer regions of Salmo-Trutta by C-, Ag-, and restriction endonuclease banding. Cytogenet Cell Genet 1990; 54(1-2): 6-9.
18. Wickstrom D, Wagner S, Baars L, et al. Consequences of depletion of the signal recognition particle in *Escherichia coli*. J Biol Chem 2011; 286(6): 4598-4609.
19. Ding Y, Fujimoto LM, Yao Y, et al. Solid-state NMR of the *Yersinia pestis* outer membrane protein Ail in lipid bilayer nanodiscs sedimented by ultracentrifugation. J Biomol NMR 2015; 61(3-4): 275-286.
20. Kobayashi A, Kobayashi Y, Shiomi K. Fish allergy in patients with parvalbumin-specific immunoglobulin E depends on parvalbumin content rather than molecular differences in the protein among fish species. Biosci Biotechnol Biochem 2016; 80(10): 2018-2021.
21. Altun S, Adiloglu A, Kubilay A, et al. Immunogenic and antigenic profiles of nine *Lactococcus garvieae* strains from different rainbow trout farms. Isr J Aquacult-Bamid 2007; 59(2): 111-116.
22. Huang HY, Chen YC, Wang PC, et al. Efficacy of a formalin-inactivated vaccine against *Streptococcus iniae* infection in the farmed grouper *Epinephelus coioides* by intraperitoneal immunization. Vaccine 2014; 32(51): 7014-7020.
23. Winkelstroter LK, Tulini FL, De Martinis ECP. Identification of the bacteriocin produced by cheese isolate *Lactobacillus paraplantarum* FT259 and its potential influence on Listeria monocytogenes biofilm formation. Lwt-Food Sci Technol 2015; 64(2): 586-592.
24. Hunter PR, Gaston MA. Numerical Index of the discriminatory ability of typing systems - an application of simpsons index of diversity. J Clin Microbiol 1988; 26(11): 2465-2466.
25. Descheemaeker P, Pot B, Ledeboer AM, et al. Comparison of the *Lactococcus lactis* differential medium (Dcl) and SDS-Page of whole-cell proteins for the identification of Lactococci to subspecies level. Syst Appl Microbiol 1994; 17(3): 459-466.
26. Dos Santos O, De Resende MCC, De Mello AL, et al. The use of whole-cell protein profile analysis by SDS-PAGE as an accurate tool to identify species and subspecies of coagulase-negative staphylococci. APMIS 2012; 120(1): 39-46.
27. Raissy M, Ansari M. Antibiotic susceptibility of *Lactococcus garvieae* isolated from rainbow trout (Oncorhynchus mykiss) in Iran fish farms. Afr J Biotechnol 2011; 10(8): 1473-1476.
28. Ture M, Boran H. Phenotypic and genotypic antimicrobial resistance of *Lactococcus* sp, strains isolated from rainbow trout (*Oncorhynchus mykiss*). Bull Vet Inst Pulawy 2015; 59: 37-42.
29. Madureira P, Baptista M, Vieira M, et al. Streptococcus agalactiae GAPDH is a virulence-associated immunomodulatory protein. J Immunol 2007; 178(3): 1379-1387.
30. Tsai MA, Wang PC, Cao TT, et al. Immunoprotection of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from *Lactococcus garvieae* against Lactococcosis in tilapia. J Gen Appl Microbiol 2013; 59(6): 437-449.

www.SID.ir