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REVIEW

Lactococcus garvieae: an emerging bacterial pathogen of fish

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ABSTRACT: *Lactococcus garvieae* is the causative agent of lactococcosis, a hyperacute, haemorrhagic septicaemia of fish. This bacterium is also considered an emerging zoonotic pathogen, as reports of human infection are increasing. Significant economic loss in aquaculture is suffered as a result of lactococcosis, as numerous freshwater and marine species of commercial interest are affected. Development of antibiotic resistance in *L. garvieae* to several chemotherapeutic agents complicates and restricts treatment options. Effective, sustainable treatment and prevention options are thus needed, but progress is impeded by the lack of knowledge concerning several aspects of the disease and the pathogen. This review aims to present the latest research on *L. garvieae*, with specific focus on pathogenesis, virulence factors, risks associated with chemotherapeutic administration and possible control options.

KEY WORDS: *Lactococcus garvieae* · Aquaculture · Vaccination · Antibiotic resistance · Virulence factors

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INTRODUCTION

Increasing pressure is being placed on aquaculture as the increasing demand for seafood products cannot be satisfied solely by wild fisheries. In the past century, an acceleration in the expansion of aquaculture as an industry has been observed and, as the fastest growing agricultural sector worldwide, the aquaculture industry is currently responsible for the production of 50% of food fish worldwide. Despite these demands, quality standards need to be maintained. Infectious disease caused by viruses, bacteria, protozoa and trematodes cause severe fiscal loss in aquaculture (Austin & Austin 2012). Since the initial description of a Gram-positive coccus implicated in septicaemia in rainbow trout *Oncorhynchus mykiss* (Walbaum) in Japan (Hoshino et al. 1958), the number of reports of streptococcal isolates associated with fish disease has increased worldwide (Boomker et al. 1979, Wallbanks et al. 1990, Toranzo et al. 1994, Michel et al. 1997). Based on phenotypic similarities, etiological agents of these diseases were initially assigned to the genus *Streptococcus.* Advancements in genotyping methods allowed their reclassification into the separate genera *Enterococcus* (Kusuda et al. 1991), *Vagococcus* (Wallbanks et al. 1990, Michel et al. 1997), *Carnobacterium* (Wallbanks et al. 1990) and *Lactococcus* (Doménech et al. 1993, Eldar et al. 1996). Presently, it is believed that certain species (*Vagococcus salmoninarum* and *Lactococcus piscium*) cause streptococcosis solely in salmonid fish when the water temperature is below 15°C, while other species are responsible for streptococcal outbreaks in cultured freshwater and marine fish when the water temperature rises above 15°C (Eldar & Ghittino 1999). This review concerns a bacterium, *L. garvieae*, grouped in the latter category.

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LACTOCOCCOSIS IN AQUACULTURE

Symptoms and clinical signs

Lactococcosis is defined as a systemic hyperacute infection with the occurrence of widespread haemorrhaging (Austin & Austin 2012). The earliest symptoms of infection include anorexia, melanosis and erratic swimming. Other external signs include uni- or bilateral exophthalmia, swollen abdomen and anal prolapsus (Eldar & Ghittino 1999, Bekker et al. 2011). At necropsy, accumulation of ascitic fluid in the peritoneal cavity, congestion of internal organs, enlargement of spleen and liver, and exudate covering the brain are observed (Bragg & Broere 1986, Eldar & Ghittino 1999). During macroscopic examination, extensive haemorrhaging is commonly observed, caused by injury to the vascular epithelium that leads to haemorrhages and petechiae on the surfaces of internal organs and external surfaces (Bragg & Broere 1986, Eldar & Ghittino 1999, Vendrell et al. 2006). It is likely that these clinical findings are caused by toxin production, as Kusuda & Hamaguchi (1988) have shown that symptoms can be reproduced in fish upon inoculation with extracellular products of *Lactococcus garvieae*.

Host range

The causative agent of lactococcosis, *L. garvieae*, has been isolated from a wide range of fish species (listed in Table 1). Apart from the reputation of *L. garvieae* as a fish pathogen contributing to economic losses, the involvement of *L. garvieae* in human clinical infections has been well documented (Chan et al. 2011). An increasing number of human infections due to *L. garvieae* has been reported in recent years, where handling and ingestion of raw fish is reported as a source or risk factor in the majority of clinical cases (Gibello et al. 2016), giving rise to the status of an emerging zoonotic pathogen. However, foodborne transmission of *L. garvieae* from aquatic products is mostly deduced from patients' clinical histories and isolates are often not further characterised on a molecular level. Thus far, only 2 reports confirming the association between a clinical case of *L. garvieae* infection and ingestion of raw seafood have been published (Wang et al. 2007, Tsai et al. 2012). The possibility exists that strains of piscine origin are responsible for human infections, but the data available to support this claim is insufficient. Whole-genome sequencing of *L. garvieae* isolates has revealed putative cell surface adhesin genes present in

Table 1. Aquatic hosts of *Lactococcus garvieae*

Host	Area	Reference
Japanese eel *Anguilla japonica* (Temminck & Schlegel)	Japan	Kusuda et al. (1991)
Red sea wrasse *Coris aygula* (Lacépède)	Israel	Colorni et al. (2003)
Brazil Nile tilapia *Oreochromis niloticus* L.	Brazil	Evans et al. (2009)
Pintado *Pseudoplathystoma corruscans* (Spix & Agassiz)	Brazil	Evans et al. (2009)
Olive flounder *Paralichthys olivaceous* (Temminck & Schlegel)	Japan	Kawanishi et al. (2006)
Amberjack *Seriola dumerili* (Risso)	Japan	Kawanishi et al. (2006)
Kingfish *Seriola quinqueradiata* (Temminck & Schlegel)	Japan	Kawanishi et al. (2006)
Rainbow trout *Oncorhynchus mykiss* (Walbaum)	South Africa	Boomker et al. (1979)
	Australia	Bragg & Broere (1986)
	UK	Carson et al. (1993), Bark & McGregor (2001)
	Taiwan	Chang et al. (2002)
	France	Eyngor et al. (2004)
	Bulgaria, Israel, Portugal	Pereira et al. (2004)
	Greece	Savvidis et al. (2007)
	Iran	Sharifiyazdi et al. (2010)
	Spain	Aguado-Urda et al. (2011a)
	Italy	Reimundo et al. (2011)
	Turkey	Didinen et al. (2014)
Grey mullet *Mugil cephalus* L.	Taiwan	Chen et al. (2002)
Catfish *Silurus glanis* L.	Italy	Ravelo et al. (2003)
Freshwater prawn *Macrobrachium rosenbergii* (De Man)	Taiwan	Chen et al. (2001)
Bottlenose dolphin *Tursiops truncatus* (Montagu)	Kuwait	Evans et al. (2006)
Common octopus *Octopus vulgaris* (Cuvier)	Italy	Fichi et al. (2015)

fish isolates, but absent in human isolates (Miyauchi et al. 2012). These findings may explain the observed variation in host specificity (Türe & Altinok 2016) and may demonstrate the adaptation of specific strains to a specific host, refuting the hypothesis of foodborne transmission of *L. garvieae.* Additional investigation is needed to clarify the link between aquatic outbreaks and human infections.

LACTOCOCCUS GARVIEAE

Lactococcus garvieae is a pathogen of importance in the aquaculture of freshwater and marine fish (Collins et al. 1983, Bragg & Broere 1986, Kusuda et al. 1991, Eldar et al. 1996). Initially named *Streptococcus garvieae*, it was originally isolated from a case of bovine mastitis in the UK and this isolate was selected as the reference strain (ATCC 43921) for this species (Collins et al. 1983).

Lactic streptococci in the genus *Streptococcus* were assigned to a new genus, *Lactococcus*, in 1985 (Schleifer et al. 1985). Gram-positive fish pathogens isolated from streptococcal disease outbreaks in Japanese yellowtail *Seriola quinqueradiata* were later unified under a new species, *Enterococcus seriolicida* (Kusuda et al. 1991). In 1988, a bacterium isolated from the first Spanish lactococcosis outbreak in rainbow trout was described as an *Enterococcus* sp. (Palacios et al. 1993), but was later identified as *L. garvieae* based on biochemical characteristics (Teixeira et al. 1996). South African Gram-positive cocci, initially described as *Streptococcus* spp. (Bragg & Broere 1986) were recently reclassified as *Enterococcus* spp. and *L. garvieae* based on 16S ribosomal RNA (rRNA) sequencing (Bekker et al. 2011). Recent advances in next generation sequencing technologies have contributed to a steady increase in the numbers of publically available full and partial genome sequences of *L. garvieae* over the last decade, as described in Table 2.

Phenotypic and biochemical characteristics

L. garvieae is a Gram-positive, facultative anaerobic, non-motile bacterium that does not produce endospores. Growth occurs as cocci in short chains and pairs at temperatures ranging from 4 to 45°C. Optimal growth occurs at 37°C (Boomker et al. 1979, Kusuda et al. 1991, Eldar et al. 1996). The bacterium grows fast in rich media such as trypticase−soy broth, bile−esculin agar, and brain−heart infusion broth, but growth is inhibited on McConkey and *Enterococcus* agar (Toranzo et al. 1994). It is generally described as an α-haemolytic bacterium (Ravelo et al. 2001), but has been noted as β-haemolytic (Teixeira et al. 1996). The phenotypic, physiological and biochemical properties of *L. garvieae* are listed in Table 3.

Isolation and identification

As methods for the selective isolation of *Streptococcus* spp. had not been successfully applied to the isolation of fish-pathogenic streptococcal bacteria (Bragg et al. 1989), a biphasic procedure for the selective isolation of a fish-pathogenic *Streptococcus* sp. was developed by Bragg et al. (1989). During a selective enrichment phase, environmental samples from freshwater trout farms in South Africa were

Table 2. *Lactococcus garvieae* whole genome sequences available on the NCBI database (www.ncbi.nlm.nih.gov/)

Strain	Source	Geographical origin	Accession no.	Reference
21881	Human blood	Spain	AFCC00000000	Aguado-Urda et al. (2011b)
8831	Rainbow trout	Spain	AFCD00000000	Aguado-Urda et al. (2011a)
ATCC 49156	Yellowtail	Japan	AP009332	Morita et al. (2011)
Lg2	Yellowtail	Japan	AP009333	Morita et al. (2011)
UNIUD074	Rainbow trout	Italy	AFHF00000000	Reimundo et al. (2011)
DCC43	Mallard duck intestines	Norway	AMQS00000000	Gabrielsen et al. (2012)
IPLA 31405	Raw-milk cheese	Spain	AKFO01000000	Flórez et al. (2012)
LG9	Rainbow trout	Italy	AGQY00000000	Ricci et al. (2012)
TB25	Cheese	Italy	AGQX00000000	Ricci et al. (2012)
I113	Pork sausage	Italy	AMFD00000000	Ricci et al. (2013)
Tac2	Turkey meat	Italy	AMFE00000000	Ricci et al. (2013)
122061	Yellowtail	Japan	AP017373	Nishiki et al. (2016)
PAQ102015-99	Rainbow trout	USA	LXWL00000000.	Nelson et al. (2016)

Table 3. Phenotypic characteristics of *Lactococcus garvieae* (Vendrell et al. 2006). TSI: triple sugar iron; V: variable reaction; (): weak or slow reaction; A/A–: acidification of medium

Property	Reaction	Property	Reaction
Cell morphology	Ovoid cocci	**Production of:**	
Gram stain	+	Arginine	+
Motility	–	Ornithine	–
		Lysine	–
Growth:	+		
4°C	+	**Acid from:**	
20°C	+	Glycerol	–
37°C	+	Raffinose	–
45°C	+	Arabinose	–
pH 9.6	+	Sorbitol	+
6.5 % NaCl	+	Mannitol	+
Haemolysis	α–	Cellobiose	+
Catalase	–	Galactose	+
Oxidase	–	D-Glucose	+
TSI	A/A–	Maltose	+
Oxidative/ fermentative	F	Trehalose	+
		D-Mannose	+
Nitrate reduction	–	Inositol	–
Citrate	–	Lactose	(+)
Urea	–	Ribose	V
Indole production	–	Sucrose	V
Esculin	+	Adonitol	–
Voges-Proskauer	+	Glycogen	–
H_2S production	–	Melibiose	–
Arginine dihydrolase	+	Melezitose	–
Pyrrolidonyl arylamidase	+	Starch	–
		Tagatose	V
Alkaline phosphatase	–	L-Rhamnose	–
β-Glucuronidase	V	D-Xylose	–
Leucine arylamidase	+	Salicin	+
Sodium hippurate hydrolysis	–		

inoculated into nutrient broth (pH 9.6) supplemented with nalidixic acid (160 µg ml^{-1}), followed by incubation at room temperature for 48 h (Bragg et al. 1989). The increased pH served to inhibit the growth of yeast. In the isolation phase, growth was plated onto tetrazolium agar (1.4 % m/v agar, 1 % m/v peptone, 1 % m/v lab-lemco, 0.5 % m/v NaCl, 1 % glucose and 0.01 % m/v tetrazolium salt) after which small red colonies were plated onto blood–tryptose agar. Colonies were further characterised by Gram staining. Biochemical identification, slide agglutination and immunofluorescent antibody tests were performed on Gram-positive cocci. This procedure was shown to detect about 2 bacteria per ml (Bragg et al. 1989).

A medium for differentiation between *L. garvieae* and other fish pathogens has recently been developed (Chang et al. 2014). The medium contains the selective agents Difco™ Oxgall (3 %) and potassium tellurite (10 ppm), which inhibit growth of most water-borne bacteria. A 9:1 tetrazolium mixture (2,3,5-triphenyltetrazolium chloride:tetrazolium blue chloride) at a concentration of 80 ppm was included to differentiate between capsulated and non-capsulated *L. garvieae* isolates. Differentiation is based on the conversion of TeO_3^{2-} to Te, which stains capsulated *L. garvieae* colonies metallic black, while reduction of triphenyltetrazolium to red triphenyl formazan results in a red halo. Colonies of capsulated isolates hence appear metallic black with a red halo.

Molecular techniques based on PCR methods have been shown to be useful in the identification of fish pathogens such as *L. garvieae*, as summarised in Table 4. An assay targeting the dihydropteroate synthase gene proved to specifically detect *L. garvieae* in diseased yellowtail *S. quinqueradiata* (Temminck & Schlegel) kidney homogenates (Aoki et al. 2000). An approach using PCR amplification of the internal transcribed spacer (ITS) region, situated between the 16S and 23S small-subunit rRNA genes of bacteria, was shown to be more specific than the previously published 16S rRNA-based approaches, in addition to being able to detect quantities as low as 2.63 pg DNA (Dang et al. 2012). Analysis of ITS sequence data from lactococci, streptococci and enterococci indicated a high degree of polymorphism, thus qualifying the ITS region as a valuable target for reliable differentiation of lactococci (Blaiotta et al. 2002, Dang et al. 2012).

Table 4. Genes targeted by PCR amplification in molecular identification of *Lactococcus garvieae*

Gene	Primer pairs	Product size (bp)	Reference
16S rDNA	pGL-1 pGL-2	1100	Zlotkin et al. (1998)
Dihydropteroate synthase	SA1B10-1-F SA1B10-1-R	709	Aoki et al. (2000)
16S rRNA	LcG-F LcG-R	252	Odamaki et al. (2011)
16S-23S rRNA	ITSLg30F ITSLg31R	290	Dang et al. (2012)

Antigenic characteristics

L. garvieae isolates are divided into 2 serotypes indistinguishable by biochemical tests (Kitao 1982). Early work on *L. garvieae* revealed a high degree of variability in the surface structure of fresh isolates subcultured on an artificial medium containing 2,3,5-triphenyltetrazolium chloride or subcultured repeatedly on Todd Hewitt agar. Surface antigen variability is evidenced by the inability of antiserum against subcultured isolates to agglutinate fresh isolates from diseased fish (Kitao 1982). The serotypes are distinguished by their ability to agglutinate serum raised against *L. garvieae.* Non-agglutinating phenotypes are designated KG– and agglutinating phenotypes KG+ (Hirono et al. 1999). Immunofluorescent staining techniques have been applied to isolates from yellowtail to show that KG+ antigens are concentrated on the cell surface only, while KG– antigens are located across the cell capsule (Okada et al. 2000). Transmission electron microscopy (TEM) revealed the presence of fimbriae on the surface of *L. garvieae* cells accompanied by capsular disruption following opsonisation with yellowtail immune serum (Ooyama et al. 1999). The antigenicity of these fimbriae has not yet been investigated.

Capsular variation is often the basis of serological differences in pathogens (Yother 2011). However, few reports on serological variation between capsulated *L. garvieae* isolates exist. Using dot blot assays with specific group polysaccharide as antigen, Eyngor et al. (2004) determined that 2 serovars or groups (SGT) can be distinguished among Mediterranean isolates: SGT I, inclusive of Italian and Israeli isolates, and SGT II, including Spanish, Greek and Bulgarian isolates. Heterogeneity among French isolates was observed, with isolates grouping with both SGT I and II. Eyngor et al. (2004) combined restriction fragment length polymorphism ribotyping with serological data, generating clear correlation between ribotypes and serovars: molecular typing displayed discriminatory ability superior to serotyping because related ribotypes could group into a single serotype. This phenomenon may be caused by strains possessing similarities in sections of their genomes encoding serotype-specific antigens while displaying a greater degree of heterogeneity in genome portions subjected to ribotyping. Changes in serovar prevalence are usually attributed to immune pressure and population dynamics, with farmed fish populations comparable to semi-closed communities. Limited selective pressure in the form of vaccination against *L. garvieae* had been imposed on the studied bacterial populations due to the fact that currently available vaccines are not wholly effective and that only a fraction of the host population is vaccinated (Eyngor et al. 2004). It can therefore be expected that capsular stability had been preserved in the endemic sites (Israel, Italy, Spain, Greece and Bulgaria). The serotypic diversity, correlating to clonal diversity, observed in French isolates is typical of areas where the pathogen has been introduced recently and disease outbreaks are infrequent (Eyngor et al. 2004). In a comparative serological study of Japanese and European capsulated and non-capsulated isolates from rainbow trout, antisera against all capsulated isolates strongly cross-reacted with all non-capsulated isolates, regardless of geographical origin (Barnes & Ellis 2004). Conversely, antisera against non-capsulated isolates did not react with any capsulated isolates. No cross-reactions of antisera against Japanese and European isolates were observed (Barnes & Ellis 2004). These serological differences could be attributed to variations in surface polysaccharide composition, assayed by agglutination with a panel of 15 lectins. Capsulated European isolates were agglutinated by concanavalin A, which specifically binds to α-D-mannose and α-D-glucose moieties, while Japanese isolates were not agglutinated by any lectins used in the study. Non-capsulated isolates were agglutinated by more lectins in comparison to the capsulated European isolates, perhaps revealing the carbohydrate diversity of the cell wall (Barnes & Ellis 2004).

Cell wall proteins are targets of immune surveillance, thereby contributing to serological differences between strains and forming the focus of vaccine development studies. A study by Hirono et al. (1999) identified antigenic proteins in a KG– strain that were immunologically detected by anti-KG– rabbit serum. The detected proteins included enzymes involved in various cellular processes. A protein with 37.1% homology to an *N*-acetylglucosamine-6-phosphate deacetylase of *Vibrio furnissi* was detected in KG– cells, but not KG+ cells (Hirono et al. 1999). Taking into account that *N*-acetylglucosamine-6-phosphate deacetylase plays a role in peptidoglycan and lipopolysaccharide synthesis in Gram-negative bacteria, it can be speculated that this protein plays a role in capsule synthesis in *L. garvieae*. Other proteins found to react with anti-KG– serum include proteins with 47.7% and 45.8% sequence homology to a processing protease of *Bacillus subtilis* and a trigger factor of *Escherichia coli*, respectively (Hirono et al. 1999). The trigger factor of *E. coli*, a peptidyl-prolyl-cis/trans-isomerase, is induced by cold shock and enhances cell viability at

low temperatures (Hesterkamp & Bukau 1996). A further investigation into the immunogenicity of KG+ and KG− cells using 2-dimensional gel electrophoresis and immunoblotting assays revealed that elongation factor G, guanine monophosphate synthetase, elongation factor thermo-unstable and adenosine triphosphate synthase reacted more intensely with rabbit anti-KG+ sera in comparison to rabbit anti-KG− sera, suggesting that these may be major specific antigens for the KG+ strain. Glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, arginine deaminase and ornithine carbamoyltransferase were also identified as common antigens in the 2 serotypes (Shin et al. 2007). Shin et al. (2009) repeated their 2007 study using olive flounder *Paralichthys olivaceus* (Temminck & Schlegel) sera instead of rabbit sera, and identified 8 antigenic protein spots reacting specifically with anti-KG− sera; however, these proteins could not be identified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry.

DISEASE CONTROL OPTIONS

Chemotherapeutic administration

Antibiotics have been widely used to control streptococcal infections in various fish (Aoki et al. 1990). Administration occurs generally via the oral route by combining antibiotics with specially formulated feed. Antimicrobial agents show strong *in vitro* activity against *Lactococcus garvieae*, but perform poorly under field conditions due to anorexia of infected fish (Bercovier et al. 1997) and possibly the ineffective metabolism of antibiotics in fish (Romero et al. 2012). Lincomycin, oxytetracycline and macrolide antibiotics (e.g. erythromycin, spiramycin, kitasamycin and josamycin) have been widely used to treat lactococcosis in cultured fish (Aoki et al. 1990, Kawanishi et al. 2005). In rainbow trout, erythromycin, oxytetracycline, amoxicillin and low-level doxycycline are mostly used to treat outbreaks of lactococcosis (Vendrell et al. 2006).

Antibiotic resistance

Dissemination of antibiotic resistance in bacteria has grown into a global public health concern, accelerated by the unregulated and injudicious administration of antibiotics in humans and animals (Heuer et al. 2009). In aquaculture, chemotherapeutic treatment has led to the emergence of resistant streptococcal fish pathogens (Aoki et al. 1990, Austin & Austin 2012). Multiple resistance is frequently encountered, referring to the occurrence of resistance to more than 1 chemotherapeutic agent in 1 isolate. The spread of antibiotic resistance genes in bacterial populations is aided by various mechanisms of horizontal gene transfer, of which plasmid-mediated transfer is the most widely documented in streptococcal fish pathogens. The first report describing antibiotic resistance in aquatic *Streptococcus* sp. grouped resistance in isolates from cultured yellowtail *Seriola quinqueradiata* from various locations in Japan into 2 categories: (1) intermediate-level resistance to macrolides, lincomycin and tetracycline, in which resistance genes were constitutively expressed and non-transferable; and (2) high-level resistance to macrolides, lincomycin and either tetracycline or chloramphenicol, in which resistance genes were inducible and transferable (Aoki et al. 1990). Those authors surmised that the antibiotic resistance determinants were located either on resistance (R) plasmids or transposons. These findings led to the characterisation of R plasmids isolated from erythromycin-, lincomycin- and oxytetracycline-resistant *L. garvieae* isolates, revealing the presence of the resistance genes *ermB* and *tet*(S) (Hirono & Aoki 2001). The gene *ermB* contributes to erythromycin resistance by target modification mediated by the production of a 23S rRNA methylase (Leclercq & Courvalin 1991), while the gene product of *tet*(S) is a ribosomal protection protein that confers tetracycline resistance (Chopra & Roberts 2001). A study by Kawanishi et al. (2005) corroborated the findings of Aoki et al. (1990) as well as Hirono & Aoki (2001) by reporting a high incidence of multiple resistance to erythromycin, lincomycin and oxytetracycline in Japanese *L. garvieae* aquatic isolates. Antimicrobial susceptibility determination of 170 *L. garvieae* isolates revealed that nearly half were simultaneously resistant to erythromycin (minimum inhibitory concentration [MIC] $\geq$ 2 µg ml^{-1}), lincomycin (MIC $\geq$ 128 µg ml^{-1}) and oxytetracycline (MIC $\geq$ 4 µg ml^{-1}) (Kawanishi et al. 2005). Additionally, all resistant isolates harboured the resistance genes *ermB* and *tet*(S). A study on resistance to chemotherapeutic substances in Japanese *L. garvieae* isolates by Maki et al. (2008) revealed that 31.5% of tested isolates were highly resistant (MIC > 400 µg ml^{-1}) to erythromycin, tetracycline and lincomycin. Of the highly resistant isolates, 26% carried R plasmids transferable to *Enterococcus faecalis* by conjugation (Maki et al. 2008). The remaining 74% of highly resistant isolates were shown to carry the

same resistance genes present on the R plasmid, suggesting carriage of either an integrated R plasmid or transferable low frequency plasmids. Further characterisation of the R plasmid pKL0018, described by Maki et al. (2008), revealed high sequence homology to pRE25, a plasmid found in *E. faecalis* isolated from dried sausage (Maki et al. 2009). Genes related to multiple drug resistance carried on pKL0018 were identified as the tetracycline resistance gene *tet*(S) and macrolide resistance genes encoding 23S rRNA methyltransferases (*ermB1* and *ermB2*). This work constituted the first report of the sequence of a Gram-positive bacterium-derived plasmid from a fish pathogen (Maki et al. 2009). The presence of *tet*(S) and another ribosomal protection protein gene, *tet*(M), was simultaneously detected in Japanese *L. garvieae* marine isolates (Kim et al. 2004). All but 1 of these isolates additionally harboured the integrase gene of the Tn1545–Tn916 conjugative transposon family, a first indication of horizontal transfer of resistance genes by conjugation in *L. garvieae*. The presence of transferable R plasmids and conjugative transposon-associated integrase genes in aquatic *L. garvieae* suggests that these isolates can function as antibiotic resistance vectors between clinical, terrestrial and marine environments (Kim et al. 2004). Chromosomally encoded resistance determinants have also been detected in *L. garvieae.* Mutations in the quinolone resistance-determining regions within the genes *gyrA* and *parC* of isolates highly resistant to old and new generation quinolones (MICs ≥ 200 µg ml^{-1}) have been described (Maki et al. 2008), as well as a pentapeptide repeat protein (LgaQnr) associated with decreased sensitivity to quinolones (Gibello et al. 2014).

Vaccination

Vaccination is considered the best option to control lactococcosis, due to the poor efficiency of chemotherapeutic agents under field conditions and the risks associated with the spread of antibiotic resistance determinants. Practices include intraperitoneal injection 1 mo prior to the water temperature increasing over 15°C, with care being taken to maintain fish in optimum health and reduce stress. For rainbow trout, vaccination is performed when fish weigh approximately 50 g and when the water temperature measures between 12 and 14°C (Vendrell et al. 2006). Autogenous formalin-inactivated vaccines against *L. garvieae* are commonly implemented, with protection of 80 to 90% observed upon intraperitoneal injection (Bercovier et al. 1997) and persisting for up to 5 mo with adjuvanted vaccines (Vendrell et al. 2006). The application of different adjuvant formulations in inactivated vaccines has been investigated. Vendrell et al. (2007) assessed the safety and efficacy of an inactivated vaccine Ichtiovac-Lg, emulsified with a mineral oil adjuvant (Aquamun), in rainbow trout. In a vaccine safety trial, an intraperitoneal injection of a double dose of vaccine (0.2 ml) administered to a treatment group resulted in 100% survival. The side effects observed in the vaccinated group during necropsy are considered acceptable by the European Pharmacopoeia. The side effects recorded were mild, localised adhesions and minor pigmentation of the visceral peritoneum and moderate adhesions between viscera. To test the efficacy of Ichtiovac-Lg, rainbow trout were intraperitoneally injected with the recommended dosage (0.1 ml) and challenged with a capsulated strain of *L. garvieae* (CLFP LG1) 29 d post-vaccination. Fish were kept at 19°C during challenge and post-vaccination to allow proliferation of the pathogen. A cumulative survival rate of 94% was reported for the vaccinated group, while a 4% cumulative survival rate was reported for the control group (Vendrell et al. 2007). Those authors believe that the observed efficacy of the vaccine might have been partially attributed to the immunostimmulatory effect of the mineral oil adjuvant. The efficacy of a bivalent vaccine against *L. garvieae* and *Aeromonas hydrophila* formulated with a non-mineral oil adjuvant (Montanide ISA 763 AVG) in rainbow trout has recently been demonstrated (Bastardo et al. 2012). During vaccination trials, fish were maintained at an average temperature of 14 ± 1°C during intraperitoneal vaccination and challenged with *A. hydrophila* and *L. garvieae* at 30 and 90 d post-vaccination, respectively. Results indicated that the non-mineral oil adjuvanted vaccine conferred higher levels of protection in comparison with a bivalent aqueous vaccine. Several studies have shown that subunit vaccines (i.e. vaccines consisting of immunogenic fractions) are capable of eliciting higher levels of protection in comparison to whole cell vaccines in fish (Ra et al. 2009, Zhou et al. 2010). Bacterial outer membrane proteins are often targets of subunit vaccine development, because their exposure on the cell surface promotes recognition by the host's immune system (Kawai et al. 2004). Despite promising results obtained with bacterial subunit vaccines in fish (Liu et al. 2005, Ra et al. 2009), reports on the development of subunit vaccines against lactococcosis are scarce. In a study by Tsai et al. (2013), an antigen common to both KG+ and KG–

L. garvieae serotypes, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Shin et al. 2009), was cloned and expressed in *Escherichia coli* BL21 (DE3). Western blotting analysis was used to show that both rabbit and tilapia antiserum reacted strongly with recombinant GAPDH. In addition, higher GAPDH-specific antibody titres were reported in tilapia immunised with recombinant GAPDH plus whole cells, in comparison to tilapia immunised with whole cells only. However, fish immunised with recombinant GAPDH alone showed a higher percentage cumulative mortality over a period of 14 d post-challenge than fish immunised with whole cells only and fish immunised with whole cells plus recombinant GAPDH. Tsai et al. (2013) partially attributed this observation to the immunostimulatory effect of peptidoglycan in the whole-cell vaccine. The duration of protection afforded by the GAPDH vaccine in comparison with a whole-cell vaccine has not been investigated.

VIRULENCE FACTORS

Toxins

Toxigenesis plays a crucial role in the pathologic processes of various Gram-positive bacteria (Barnett et al. 2015). Early studies on the toxins of a non-Lancefield *Streptococcus* sp. isolated from yellowtail (presumably *Lactococcus garvieae*) showed the presence of a haemolytic toxin in the culture supernatant (Kusuda & Hamaguchi 1988). Mortalities caused by intramuscular injection of this toxin were low (<20%), but characteristic symptoms of streptococcosis (i.e. exophthalmus, ocular haemorrhaging and reddish fin base) were elicited. An intracellular toxin showing weak leukocidal activity (<44%) was responsible for higher mortality rates of up to 60% upon intramuscular injection (Kusuda & Hamaguchi 1988).

A study by Aguado-Urda et al. (2012) identified and characterised 5 circular plasmids in a clinical isolate of *L. garvieae* strain 21881. The largest of these plasmids (68798 bp), pGL5, was shown to encode putative virulence factors, including a protein that possesses the enzymatic domain corresponding to the family of actin-ADP-ribosyltransferases (Aguado-Urda et al. 2012). Bacterial ADP-ribosyltransferase toxins kill eukaryotic cells by transferring ADP-ribose to essential proteins, contributing to virulence in a range of pathogens (Holbourn et al. 2006). However, toxicity of this protein remains to be demonstrated experimentally.

Immune evasion mechanisms

It has long been known that virulence of *L. garvieae* is largely influenced by capsule formation (Vendrell et al. 2006). Encapsulation contributes to virulence in both Gram-positive and Gram-negative bacteria in a number of ways, for example by conferring resistance to phagocytosis (Musher 1992) and exhibiting molecular mimicry of host tissue (Johnson 1991). Even though the polysaccharide capsule is widely regarded as a major virulence factor of *L. garvieae*, Türe et al. (2004) reported that the non-capsulated strains Lgper and ATCC 49156 are pathogenic to rainbow trout, causing 98% and 90% mortality, respectively. However, ATCC 49156 was shown to be less virulent by immersion exposure in comparison to intraperitoneal injection (12% and 90% disease related mortality, respectively) (Türe et al. 2014). Detection of putative virulence genes in 34 fish-pathogenic *L. garvieae* isolates revealed that the capsule gene cluster could only be amplified by multiplex PCR in the strain Lg2 (Türe & Altinok 2016). These results suggest that the presence of the polysaccharide capsule cannot be directly correlated to pathogenicity in fish. A comparative genome analysis of the virulent strain Lg2 and sATCC 49156 (previously believed to be avirulent toward fish) identified a 16.5 kb capsule gene cluster which is present in Lg2, but absent in ATCC 49156 (Morita et al. 2011). The capsular gene cluster consists of 15 genes, of which 8 (*eps-R*, *-X*, *-A*, *-B*, *-C*, *-D* and *cps-L*, *-W*) are conserved in the exopolysaccharide (EPS) biosynthesis gene cluster of 4 *L. lactis* strains isolated from human faecal samples (Morita et al. 2011). Analyses indicate that the capsular gene cluster is a genomic island, due to the presence of insertion sequences on both ends of the capsular gene cluster.

The KG− phenotype of *L. garvieae* possesses a capsule rich in hydrophilic monosaccharides, possibly contributing to an observed increased resistance to phagocytosis by *Seriola quinqueradiata* phagocytes in comparison to KG+ cells: the respiratory burst in phagocytic cells was suppressed in response to the KG− phenotype, indicating inhibition of the binding of phagocytes to the encapsulated strain (Yoshida et al. 1996). These *in vitro* findings are supported by challenge studies that indicate lower serum agglutinating antibody titres in fish challenged with KG− cells in comparison to KG+ cells (Yoshida et al. 1996). Noncapsulated *L. garvieae* isolated from radish and broccoli sprouts were found to be nonpathogenic toward mice and yellowtail. In addition, the avirulence of non-capsulated isolates correlated with their sus-

ceptibility to rainbow trout normal serum, while capsulated isolates were not susceptible to either normal or immune rainbow trout serum (Barnes et al. 2002a). Protection against capsulated isolates was afforded by passive immunisation of rainbow trout with specific antiserum against *L. garvieae*, leading those authors to speculate that specific antibodies enhance phagocytosis and bactericidal activity by macrophages (Barnes et al. 2002a). They did indeed show that the anti-phagocytic properties of the polysaccharide capsule can be overcome in the presence of specific antibodies (Barnes et al. 2002a). The observed increased bactericidal activity of immune serum was likely not due to complement, as the serum was heat-treated. Fluorescence microscopy of fluorescein-isothiocyanate labelled bacteria incubated with macrophages indicated that 90 % of macrophages contained internalised bacteria treated with immune serum, while between 0 and 2 % of macrophages internalised bacteria treated with non-immune serum (Barnes et al. 2002b). Many Gram-positive bacteria employ non-specific binding of immunoglobulins by their crystallizable fragment region as a virulence factor (Agniswamy et al. 2004). Binding of antibodies by non-immune mechanisms inhibits activation of complement by the classical pathway and allows the bacterium to shield itself from specific antibodies and evade phagocytosis. Barnes et al. (2002b) showed that non-capsulated *L. garvieae* was capable of non-specifically binding immunoglobulin more efficiently than capsulated isolates, an observation which seems inconsistent with the avirulence of non-capsulated isolates. However, it must be considered that surface proteins play integral roles in adhesion and colonisation of host tissues, and that non-specific binding to host serum proteins might therefore inhibit adhesion of non-capsulated isolates to host cells (Barnes et al. 2002a). Interestingly, capsulated *L. garvieae* Lg2 has been shown to be avirulent toward mice (Kawanishi et al. 2007) and *L. garvieae* 21881 (isolated from blood of a septicaemic patient) lacks a capsule gene cluster (Miyauchi et al. 2012), perhaps indicating that encapsulation is not a prerequisite for virulence in mammals.

Adhesion

Both commensal and pathogenic bacteria express adhesins to facilitate binding to host cell receptors (Kline et al. 2009). Genes encoding 2 putative surface proteins that contain a cell wall sorting motif associated with covalent binding to peptidoglycan, LPXTG (Leu-Pro-any-Thr-Gly), have been identified on the plasmid pGL5 of a clinical *L. garvieae* isolate (21881). The gene *orf5* encodes a protein containing 3 mucin-binding protein domains in addition to a cell wall sorting motif (LPQTG) at the carboxy terminal, suggesting that protein Orf5 might aid in adhesion of *L. garvieae* to mucosa by interaction with mucosal receptors (Aguado-Urda et al. 2012). Another putative cell surface protein, encoded by *orf25*, contains a collagen-binding domain which could allow adhesion of the cell to collagenous host tissues.

Based on the work of Miyauchi et al. (2012), Türe & Altinok (2016) determined the prevalence of a variety of putative virulence genes among 34 fish-pathogenic *L. garvieae* isolates by PCR. The adhesin-encoding genes adhesin Pav (*adh*Pav) and adhesin PsaA (*adh*PsaA), LPxTG-containing surface proteins 2 and 3 (LPxTG-2, -3) and adhesin clusters 1 and 2 (*adh*CI, *adh*CII) were present in all isolates tested.

Diversification of virulence factor content

Insertion sequences, plasmids and lysogenic bacteriophages are mobile genetic elements which play an important evolutionary role by promoting adaptability in prokaryotic genomes (Eraclio et al. 2015). During horizontal gene transfer, insertion sequences can play an important role in bacterial pathogenesis and exchange of virulence factors. Fifteen insertion sequences have been identified in the publically available genomes of *L. garvieae* (Eraclio et al. 2015). The close relatedness described between insertion sequences in *L. garvieae* and *L. lactis* may suggest genetic exchange between the species.

Bacteriophages are viruses that infect and kill bacterial cells with great efficacy and are present in all ecosystems that support the growth of bacteria (Elbreki et al. 2014). The presence of a lysogenic bacteriophage (PLgT-1) in the genomes of *L. garvieae* strains isolated from Japanese marine fish species has recently been discovered (Hoai & Yoshida 2016). Bacteriophages were induced by mitomycin C treatment, integrated genomes of bacteriophages (prophages) were detected by a PCR assay and morphological study of the phage particles by TEM revealed characteristics congruent with the morphology of phages from the family *Siphoviridae.* Considering the high incidence of prophages in strains isolated from Japanese marine fish revealed by this study in conjunction with the high virulence of marine isolates compared to trout and terrestrial mammalian isolates (Kawanishi et al.

2006), it is thus probable that prophages contribute to the virulence of *L. garvieae.*

CONCLUSION

Lactococcosis is a globally occurring disease of cultured fish caused by a Gram-positive bacterium *Lactococcus garvieae*. In addition to causing monetary damage to the ever-growing aquaculture industry globally, it is considered an emerging zoonotic agent placing immunocompromised individuals at risk (Gibello et al. 2016). Application of chemotherapeutic agents is effective under experimental conditions, but is ultimately an unsustainable strategy in the control of lactococcosis due to the development and spread of antibiotic resistance. Many reports concerning high levels of multiple resistance to frequently used antibiotics (macrolides, lincomycin and tetracycline) exist, creating the need to investigate alternatives for disease prevention and control. Preventative measures in the form of biosecurity and vaccination can be investigated as alternatives to antibiotic dependency. The possible application of new generation vaccines for the prevention of lactococcosis is yet to be fully investigated.

Although putative adhesion factors have been identified in a clinical *L. garvieae* isolate, this integral step in the infection process has not been further elucidated. The polysaccharide capsule of *L. garvieae* has been widely described as a major virulence factor involved mainly in the evasion of the host immune response, although none has been structurally characterised. Serologically differing, capsulated, geographically distinct isolates have been shown to possess differences in capsular carbohydrate composition, suggesting that EPS structure may form the basis of serological variability as observed in various Gram-positive pathogens. Understanding of the underlying genetic basis of variability in EPS structure and its likely interrelationship with serological variability in *L. garvieae* is currently lacking. Recent findings suggest that the EPS capsule is not the sole determinant of pathogenicity and various virulence factors are involved in the pathogenesis of *L. garvieae.* Lack of knowledge of virulence factors, pathogenesis and serology of *L. garvieae* is an impediment to the development of effective typing methods and control measures.

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LITERATURE CITED

Agniswamy J, Lei B, Musser JM, Sun PD (2004) Insight of host immune evasion mediated by two variants of group A *Streptococcus* Mac protein. J Biol Chem 279:52789–52796

Aguado-Urda M, López-Campos GH, Blanco MM, Fernández-Garayzábal JF and others (2011b) Genome sequence of *Lactococcus garvieae* 21881, isolated in a case of human septicemia. J Bacteriol 193:4033–4034

Aguado-Urda M, López-Campos GH, Gibello A, Cutuli MT, López-Alonso V, Fernández-Garayzábal JF, Blanco MM (2011a) Genome sequence of *Lactococcus garvieae* 8831, isolated from rainbow trout lactococcosis outbreaks in Spain. J Bacteriol 193:4263–4264

Aguado-Urda M, Gibello A, Blanco MM, López-Campos GH, Cutuli MT, Fernández-Garayzábal JF (2012) Characterization of plasmids in a human clinical strain of *Lactococcus garvieae.* PLOS ONE 7:e40119

Aoki T, Takami K, Kitao T (1990) Drug resistance in a non-hemolytic *Streptococcus* sp. isolated from cultured yellowtail *Seriola quinqueradiata.* Dis Aquat Org 8:171–177

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

Austin B, Austin AD (2012) Bacterial fish pathogens: Diseases of farmed and wild fish, 5th edn. Springer/Praxis Publishing, Chichester

Bark S, McGregor D (2001) The first occurrence of lactococcosis in farmed trout in England. Trout News 31:9–11

Barnes AC, Ellis AE (2004) Role of capsule in serotypic differences and complement fixation by *Lactococcus garvieae.* Fish Shellfish Immunol 16:207–214

Barnes AC, Guyot C, Hansen BG, Mackenzie K, Horne MT, Ellis AE (2002a) Resistance to serum killing may contribute to differences in the abilities of capsulate and non-capsulated isolates of *Lactococcus garvieae* to cause disease in rainbow trout (*Oncorhynchus mykiss* L.). Fish Shellfish Immunol 12:155–168

Barnes AC, Guyot C, Hansen BG, Horne MT, Ellis AE (2002b) Antibody increases phagocytosis and killing of *Lactococcus garvieae* by rainbow trout (*Oncorhynchus mykiss,* L.) macrophages. Fish Shellfish Immunol 12: 181–186

Barnett TC, Cole JN, Rivera-Hernandez T, Henningham A, Paton JC, Nizet V, Walker MJ (2015) Streptococcal toxins: role in pathogenesis and disease. Cell Microbiol 17: 1721–1741

Bastardo A, Ravelo C, Castro N, Calheiros J, Romalde JL (2012) Effectiveness of bivalent vaccines against *Aeromonas hydrophila* and *Lactococcus garvieae* infections in rainbow trout *Oncorhynchus mykis*s (Walbaum). Fish Shellfish Immunol 32:756–761

Bekker A, Hugo C, Albertyn J, Boucher CE, Bragg RR (2011) Pathogenic Gram-positive cocci in South African rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 34:483–487

Bercovier H, Ghittino C, Eldar A (1997) Immunization with bacterial antigens: infections with streptococci and related organisms. Dev Biol Stand 90:153–160

Blaiotta G, Pepe O, Mauriello G, Villani F, Andolfi R, Moschetti G (2002) 16S–23S rDNA intergenic spacer region polymorphism of *Lactococcus garvieae, Lactococcus raffinolactis* and *Lactococcus lactis* as revealed by PCR and nucleotide sequence analysis. Syst Appl Microbiol 25:520–527

Boomker J, Imes G, Cameron C, Naudé T, Schoonbee H (1979) Trout mortalities as a result of *Streptococcus* infection. Onderstepoort J Vet Res 46:71–77

Bragg RR, Broere JS (1986) Streptococcosis in rainbow trout in South Africa. Bull Eur Assoc Fish Pathol 6:89–91

Bragg RR, Todd JM, Lordan SM, Combrink ME (1989) A selective procedure for the field isolation of pathogenic *Streptococcus* spp. of rainbow trout (*Salmo gairdneri*). Onderstepoort J Vet Res 56:179–184

Carson J, Gudkovs N, Austin B (1993) Characteristics of an *Enterococcus*-like bacterium from Australia and South Africa, pathogenic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 16:381–388

Chan JFW, Woo PCY, Teng JLL, Lau SKP, Leung SSM, Tam FCC, Yuen KY (2011) Primary infective spondylodiscitis caused by *Lactococcus garvieae* and a review of human *L. garvieae* infections. Infection 39:259–264

Chang CI, Lee CF, Tsai JM, Wu CC, Chen LH, Chen SC, Lin KJ (2014) Development of a selective and differential medium for capsulated *Lactococcus garvieae.* J Fish Dis 37:719–728

Chang PH, Lin CW, Lee YC (2002) *Lactococcus garvieae* infection of cultured rainbow trout, *Oncorhynchus mykiss*, in Taiwan and associated biophysical characteristics and histopathology. Bull Eur Assoc Fish Pathol 22: 319–327

Chen SC, Lin YD, Liaw LL, Wang PC (2001) *Lactococcus garvieae* infection in the giant freshwater prawn *Macrobranchium rosenbergii* confirmed by polymerase chain reaction and 16S rDNA sequencing. Dis Aquat Org 45: 45–52

Chen SC, Liaw LL, Su HY, Ko SC and others (2002) *Lactococcus garvieae*, a cause of disease in grey mullet, *Mugil cephalus* L., in Taiwan. J Fish Dis 25:727–732

Chopra I, Roberts M (2001) Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev 65: 232–260

Collins MD, Farrow JAE, Phillips BA, Kandler O (1983) *Streptococcus garvieae* sp. nov. and *Streptococcus plantarum* sp. nov. J Gen Microbiol 129:3427–3431

Colorni A, Ravelo C, Romalde JL, Toranzo AE, Diamant A (2003) *Lactococcus garvieae* in wild Red Sea wrasse *Coris aygula* (Labridae). Dis Aquat Org 56:275–278

Dang HT, Park HK, Myung SC, Kim W (2012) Development of a novel PCR assay based on the 16S-23S rRNA internal transcribed spacer region for the detection of *Lactococcus garvieae.* J Fish Dis 35:481–487

Didinen BI, Yardimci B, Onuk EE, Metin S, Yildirim P (2014) Naturally *Lactococcus garvieae* infection in rainbow trout (*Oncorhyncus mykiss* Walbaum, 1792): new histopathological observations, phenotypic and molecular identification. Rev Med Vet (Toulouse) 165:12–19

Doménech A, Prieta J, Fernández-Garayzábal JF, Collins MD, Jones D, Domínquez L (1993) Phenotypic and phylogenetic evidence for a close relationship between *Lactococcus garvieae* and *Enterococcus seriolicida.* Microbiologia 9:63–68

Elbreki M, Ross RP, Hill C, O'Mahony J, McAuliffe O, Coffey A (2014) Bacteriophages and their derivatives as biotherapeutic agents in disease prevention and treatment. J Viruses 2014:382539

Eldar A, Ghittino C, Asanta L, Bozzetta E, Goria M, Prearo M, Bercovier H (1996) *Enterococcus seriolicida* is a junior synonym of *Lactococcus garvieae*, a causative agent of septicemia and meningoencephalitis in fish. Curr Microbiol 32:85–88

Eldar A, Ghittino C (1999) *Lactococcus garvieae* and *Streptococcus iniae* infections in rainbow trout *Oncorhynchus mykiss*: similar, but different diseases. Dis Aquat Org 36:227–231

Eraclio G, Ricci G, Fortina MG (2015) Insertion sequence elements in *Lactococcus garvieae.* Gene 555:291–296

Evans JJ, Pasnik DJ, Klesius PH, Al-Ablani S (2006) First report of *Streptococcus agalactiae* and *Lactococcus garvieae* from a wild bottlenose dolphin (*Tursiops truncatus*). J Wildl Dis 42:561–569

Evans JJ, Klesius PH, Shoemaker CA (2009) First isolation and characterization of *Lactococcus garvieae* from Brazilian Nile tilapia, *Oreochromis niloticus* (L.), and pintado, *Pseudoplathystoma corruscans* (Spix & Agassiz). J Fish Dis 32:943–951

Eyngor M, Zlotkin A, Ghittino C, Prearo M, Douet DG, Chilmonczyk S, Eldar A (2004) Clonality and diversity of the fish pathogen *Lactococcus garvieae* in Mediterranean countries. Appl Environ Microbiol 70:5132–5137

Fichi G, Cardeti G, Perrucci S, Vanni A and others (2015) Skin lesion-associated pathogens from *Octopus vulgaris*: first detection of *Photobacterium swingsii*, *Lactococcus garvieae* and betanodavirus. Dis Aquat Org 115: 147–156

Flórez AB, Reimundo P, Delgardo S, Fernández E, Alegría Á, Guijarro JA, Mayo B (2012) Genome sequence of *Lactococcus garvieae* IPLA 31405, a bacteriocin-producing, tetracycline-resistant strain isolated from a raw-milk cheese. J Bacteriol 194:5118–5119

Gabrielsen C, Brede DA, Hernández PE, Nes IF, Diepa DB (2012) Genome sequence of the bacteriocin-producing strain *Lactococcus garvieae* DCC43. J Bacteriol 194: 6976–6977

Gibello A, Díaz de Alba P, Blanco MM, Machuca J, Cutuli MT, Rodríguez-Martínez JM (2014) *Lactococcus garvieae* carries a chromosomally encoded pentapeptide repeat protein that confers reduced susceptibility to quinolones in *Escherichia coli* producing a cytotoxic effect. Res Microbiol 165:590–599

Gibello A, Galán-Sánchez F, Blanco MM; Rodríguez-Iglesias M, Domínguez L, Fernández-Garayzábal JF (2016) The zoonotic potential of *Lactococcus garviea*: an overview on microbiology, epidemiology, virulence factors and relationship with its presence in foods. Res Vet Sci 109:59–70

Hesterkamp T, Bukau B (1996) The *Escherichia coli* trigger factor. FEBS Lett 389:32–34

Heuer OE, Kruse H, Grave K, Collignon P, Karunasagar I, Angulo FS (2009) Human health consequences of use of antimicrobial agents in aquaculture. Clin Infect Dis 49: 1248–1253

Hirono I, Aoki T (2001) Characterization of structure and genes of R Plasmid from fish-pathogenic *Lactococcus garvieae.* Proc Jpn Soc Antimicrob Anim 23:22–24 (in Japanese with English Abstract)

Hirono I, Yamashita H, Park CI, Yoshida T, Aoki T (1999) Identification of genes in a KG– phenotype of *Lactococcus garvieae*, a fish pathogenic bacterium, whose proteins react with antiKG– rabbit serum. Microb Pathog 27:407–417

Hoai TD, Yoshida T (2016) Induction and characterization of a lysogenic bacteriophage of *Lactococcus garvieae* isolated from marine fish species. J Fish Dis 39:799–808

Holbourn KP, Shone CC, Acharya KR (2006) A family of killer toxins: exploring the mechanism of ADP-ribosylating toxins. FEBS J 273:4579–4593

Hoshino T, Sano T, Morimoto Y (1958) A *Streptococcus* pathogenic to fish. J Tokyo Univ Fish 44:57–58

Johnson JR (1991) Virulence factors in *Escherichia coli* urinary tract infection. Clin Microbiol Rev 4:80–128

Kawai K, Liu Y, Ohnishi K, Oshima SI (2004) A conserved 37 kDa outer membrane protein of *Edwardsiella tarda* is an effective vaccine candidate. Vaccine 22:3411–3418

Kawanishi M, Kojima A, Ishihara K, Esaki H and others (2005) Drug resistance and pulsed-field gel electrophoresis patterns of *Lactococcus garvieae* isolates from cultured *Seriola* (yellowtail, amberjack and kingfish) in Japan. Lett Appl Microbiol 40:322–328

Kawanishi M, Yoshida T, Yagashiro S, Kijima M and others (2006) 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 101:496–504

Kawanishi M, Yoshida T, Kijima M, Yagyu K and others (2007) Characterization of *Lactococcus garvieae* isolated from radish and broccoli sprouts that exhibited a KG+ phenotype, lack of virulence and absence of a capsule. Lett Appl Microbiol 44:481–487

Kim SR, Nonaka L, Suzuki S (2004) Occurrence of tetracycline resistance genes *tet*(*M*) and *tet*(*S*) in bacteria from marine aquaculture sites. FEMS Microbiol Lett 237: 147–156

Kitao T (1982) The methods for detection of *Streptococcus* sp., causative bacteria of streptococcal disease of cultured yellowtail (*Seriola quinqueradiata*), especially their cultural, biochemical and serological properties. Fish Pathol 17:17–26 (in Japanese with English abstract)

Kline KA, Fälker S, Dahlberg S, Normark S, Henriques-Normark B (2009) Bacterial adhesins in host-microbe interactions. Cell Host Microbe 5:580–592

Kusuda R, Hamaguchi M (1988) Extracellular and intracellular toxins of *Streptococcus* sp. isolated from yellowtail. Bull Eur Assoc Fish Pathol 8:9–10

Kusuda R, Kawai K, Salati F, Banner CR, Fryer JL (1991) *Enterococcus seriolicida* sp. nov., a fish pathogen. Int J Syst Evol Bacteriol 41:406–409

Leclercq R, Courvalin P (1991) Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. Antimicrob Agents Chemother 35: 1267–1272

Liu Y, Oshima S, Kurohara K, Ohnishi K, Kawai K (2005) Vaccine efficacy of recombinant GAPDH of *Edwardsiella tarda* against Edwardsiellosis. Microbiol Immunol 49: 605–612

Maki T, Hirono I, Kondo H, Aoki T (2008) Drug resistance mechanism of the fish-pathogenic bacterium *Lactococcus garvieae.* J Fish Dis 31:461–468

Maki T, Santos MD, Kondo H, Hirono I, Aoki T (2009) A transferable 20-kilobase multiple drug resistance-conferring R plasmid (pKL0018) from a fish pathogen (*Lactococcus garvieae*) is highly homologous to a conjugative multiple drug resistance-conferring enterococcal plasmid. Appl Environ Microbiol 75:3370–3372

Michel C, Nougayrède P, Eldar A, Sochon E, de Kinkelin P (1997) *Vagococcus salmoninarum*, a bacterium of pathological significance in rainbow trout *Oncorhynchus mykiss* farming. Dis Aquat Org 30:199–208

Miyauchi E, Toh H, Nakano A, Tanabe S, Morita H (2012) Comparative genomic analysis of *Lactococcus garvieae* strains isolated from different sources reveals candidate virulence genes. Int J Microbiol 2012:728276

Morita H, Toh H, Oshima K Yoshizaki M and others (2011) Complete genome sequence and comparative analysis of the fish pathogen *Lactococcus garvieae.* PLOS ONE 6: e23184

Musher DM (1992) Infections caused by *Streptococcus pneumoniae*: clinical spectrum, pathogenesis, immunity, and treatment. Clin Infect Dis 14:801–809

Nelson MC, Varney JS, Welch TJ, Graf J (2016) Draft genome sequence of *Lactococcus garvieae* strain PAQ102015-99, an outbreak strain isolated from a commercial trout farm in the northwestern United States. Genome Announc 4:e00781-16

Nishiki I, Oinaka D, Iwasaki Y, Yasuike M and others (2016) Complete genome sequence of nonagglutinating *Lactococcus garvieae* strain 122061 isolated from yellowtail in Japan. Genome Announc 4:e00592-16

Odamaki T, Yonezawa S, Kitahara M, Sugahara Y and others (2011) Novel multiplex polymerase chain reaction primer set for identification of *Lactococcus* species. Lett Appl Microbiol 52:491–496

Okada T, Minami T, Ooyama T, Yasuda H, Yoshida T (2000) Capsular and non-capsular antigen localization on *Lactococcus garvieae* isolated from yellowtail *Seriola quinqueradiata.* Fish Sci 66:992–994

Ooyama T, Kera A, Okada T, Inglis V, Yoshida T (1999) The protective immune response of yellowtail *Seriola quinqueradiata* to the bacterial fish pathogen *Lactococcus garvieae.* Dis Aquat Org 37:121–126

Palacios MA, Zamora MJ, Vázquez J, Zamora E, Duran A (1993) Streptococcosis in rainbow trout (*Oncorhynchus mykiss*) in Spain. Boll Soc Ital Patol Ittica 13:11–16

Pereira F, Ravelo C, Toranzo AE, Romalde JL (2004) *Lactococcus garvieae*, an emerging pathogen for the Portuguese trout culture. Bull Eur Assoc Fish Pathol 24: 274–279

Ra CH, Kim YJ, Park SJ, Jeong CW, Nam YK, Kim KH, Kim SK (2009) Evaluation of optimal culture conditions for recombinant ghost bacteria vaccine production with the antigen of *Streptococcus iniae* GAPDH. J Microbiol Biotechnol 19:982–986

Ravelo C, Magariños B, López-Romalde S, Toranzo AE, Romalde JL (2003) Molecular fingerprinting of fish-pathogenic *Lactococcus garvieae* strains by random amplified polymorphic DNA analysis. J Clin Microbiol 41: 751–756

Ravelo C, Magariños B, Romalde JL, Toranzo AE (2001) Conventional versus miniaturized systems for the phenotypic characterization of *Lactococcus garvieae* strains. Bull Eur Assoc Fish Pathol 21:136–144

Reimundo P, Pignatelli M, Alcaraz LD, D'Auria G, Moya A, Guijarro JA (2011) Genome sequence of *Lactococcus garvieae* UNIUD074, isolated in Italy from a lactococcosis outbreak. J Bacteriol 193:3684–3685

Ricci G, Ferrario C, Borgo F, Rollando A, Fortina MG (2012) Genome sequences of *Lactococcus garvieae* TB25, isolated from Italian cheese, and *Lactococcus garvieae* LG9, isolated from Italian rainbow trout. J Bacteriol 194: 1249–1250

Ricci G, Ferrario C, Borgo F, Eraclio G, Fortina MG (2013) Genome sequences of two *Lactococcus garvieae* strains isolated from meat. Genome Announc 1:e00018-12

Romero J, Feijoó CG, Navarrete P (2012) Antibiotics in aquaculture – use, abuse and alternatives. In: Carvalho E, David GS, da Silva RJ (eds) Health and environment in aquaculture. InTech, Rijeka, p 159–198

Savvidis GK, Anatoliotis C, Kanaki Z, Vafeas G (2007) Epizootic outbreaks of Lactococcosis disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum), culture in Greece. Bull Eur Assoc Fish Pathol 27:223–228

Schleifer KH, Kraus J, Dvorak C, Kilpper-Bälz R, Collins MD, Fischer W (1985) Transfer of *Streptococcus lactis* and related streptococci to the genus *Lactococcu*s gen. nov. Syst Appl Microbiol 6:183–195

Sharifiyazdi H, Akhlaghi M, Tabatabaei M, Mostafavi Zadeh SM (2010) Isolation and characterization of *Lactococcus garvieae* from diseased rainbow trout (*Oncorhynchus mykiss,* Walbaum) cultured in Iran. Iran J Vet Res 11:342–350

Shin GW, Palaksha KJ, Kim YR, Nho SW and others (2007) Immunoproteomic analysis of capsulate and non-capsulate strains of *Lactococcus garvieae.* Vet Microbiol 119: 205–212

Shin GW, Nho SW, Park SB, Jang HB and others (2009) Comparison of antigenic proteins from *Lactococcus garvieae* KG (–) and KG (+) strains that are recognized by olive flounder (*Paralichthys olivaceus*) antibodies. Vet Microbiol 139:113–120

Teixeira LM, Merquior VLC, Vianni MCE, Carvalho MGS and others (1996) Phenotypic and genotypic characterization of atypical *Lactococcus garvieae* strains isolated from water buffalos with subclinical mastitis and confirmation of *L. garvieae* as a senior subjective synonym of *Enterococcus seriolicida.* Int J Syst Bacteriol 46:664–668

Toranzo AE, Devesa S, Heinen P, Riaza A, Núñez S, Barja JL (1994) Streptococcosis in cultured turbot caused by an *Enterococcus*-like bacterium. Bull Eur Assoc Fish Pathol 14:19–23

Tsai MA, Wang PC, Liaw LL, Yoshida T, Chen SC (2012) Comparison of genetic characteristics and pathogenicity of *Lactococcus garvieae* isolated from aquatic animals in Taiwan. Dis Aquat Org 102:43–51

Tsai MA, Wang PC, Cao TT, Liao PC, Liaw LL, Chen SC (2013) Immunoprotection of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from *Lactococcus garvieae* against lactococcosis in tilapia. J Gen Appl Microbiol 59:437–449

Ture M, Altinok I (2016) Detection of putative virulence genes of *Lactococcus garvieae.* Dis Aquat Org 119:59–66

Ture M, Haliloğlu Hİ, Altuntaş C, Boran H, Kutlu İ (2014) Comparison of experimental susceptibility of rainbow trout (*Oncorhynchus mykiss*), turbot (*Psetta maxima*), black sea trout (*Salmo trutta labrax*) and sea bass (*Dicentrarchus labrax*) to *Lactococcus garvieae.* Turk J Fish Aquat Sci. 14:507–513

Vendrell D, Balcázar JL, Ruiz-Zarzuela I, de Blas I, Gironés O, Múzquiz JL (2006) *Lactococcus garvieae* in fish: a review. Comp Immunol Microbiol Infect Dis 29:177–198

Vendrell D, Balcázar JL, Ruiz-Zarzuela I, de Blas I, Gironés O, Múzquiz JL (2007) Safety and efficacy of an inactivated vaccine against *Lactococcus garvieae* in rainbow trout (*Oncorhynchus mykiss*). Prev Vet Med 80:222–229

Wallbanks S, Martinez-Murcia AJ, Fryer JL, Phillips BA, Collins MD (1990) 16S rRNA sequence determination for *Carnobacterium* and related lactic acid and description of *Vagococcus salmoninarum* sp. nov. Int J Syst Bacteriol 40:224–230

Wang CYC, Shie HS, Chen SC, Huang JP and others (2007) *Lactococcus garvieae* infections in humans: possible association with aquaculture outbreaks. Int J Clin Pract 61:68–73

Yoshida T, Eshima T, Wada Y, Yamada Y and others (1996) Phenotypic variation associated with an anti-phagocytic factor in the bacterial fish pathogen *Enterococcus seriolicida.* Dis Aquat Org 25:81–86

Yother J (2011) Capsules of *Streptococcus pneumoniae* and other bacteria: paradigms for polysaccharide biosynthesis and regulation. Annu Rev Microbiol 65:563–581

Zhou L, Wang X, Liu Q, Wang Q, Zhao Y, Zhang Y (2010) A novel multivalent vaccine based on secretary antigen-delivery induces protective immunity against *Vibrio anguillarum* and *Aeromonas hydrophila.* J Biotechnol 146:25–30

Zlotkin A, Eldar A, Ghittino C, Bercovier H (1998) Identification of *Lactococcus garvieae* by PCR. J Clin Microbiol 36:983–985

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