Veterinary Microbiology 201 (2017) 216–224

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Genetic and serological diversity of *Flavobacterium psychrophilum* isolates from salmonids in United Kingdom

Thao P.H. Ngo[a,*], Kerry L. Bartie[a], Kim D. Thompson[a,b], David W. Verner-Jeffreys[c], Rowena Hoare[a], Alexandra Adams[a]

[a] Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, UK
[b] Moredun Research Institute, Pentlands Science Park, Penicuik, EH26 0PZ, UK
[c] The Centre for Environment, Fisheries and Aquaculture Science, The Nothe, Barrack Road, Weymouth, DT4 8UB, UK

ARTICLE INFO

Article history:
Received 14 September 2016
Received in revised form 26 January 2017
Accepted 27 January 2017

Keywords:
Rainbow trout fry syndrome
Bacterial cold water disease
Flavobacterium psychrophilum
Genotyping
Serotyping
Plasmids

ABSTRACT

Flavobacterium psychrophilum is one of the most important bacterial pathogens affecting cultured rainbow trout (*Oncorhynchus mykiss*) and is increasingly causing problems in Atlantic salmon (*Salmo salar* L.) hatcheries. Little is known about the heterogeneity of *F. psychrophilum* isolates on UK salmonid farms. A total of 315 *F. psychrophilum* isolates, 293 of which were collected from 27 sites within the UK, were characterised using four genotyping methods and a serotyping scheme. A high strain diversity was identified among the isolates with 54 pulsotypes, ten $(GTG)_5$-PCR types, two 16S rRNA allele lineages, seven plasmid profiles and three serotypes. Seven PFGE groups and 27 singletons were formed at a band similarity of 80%. PFGE group P (n = 75) was found to be numerically predominant in eight sites within the UK. Two major PFGE clusters and 13 outliers were found at the band similarity of 40%. The predominant profileobserved within the *F. psychrophilum* isolates examined was PFGE cluster II – $(GTG)_5$-PCR type r1– 16S rRNA lineage II – serotype Th (70/156 isolates examined, 45%). Co-existence of genetically and serologically heterogeneous isolates within each farm was detected, confounding the ability to control RTFS outbreaks. The occurrence over time (up to 11 years) of *F. psychrophilum* pulsotypes in three representative sites (Scot I, Scot III and Scot V) within Scotland was examined, potentially providing important epidemiological data for farm management and the development of site-specific vaccines.

1. Introduction

Flavobacterium psychrophilum, a Gram-negative, filamentous, psychrotrophic bacterium first isolated in 1948, is an important bacterial pathogen in salmonid culture industry worldwide (Borg, 1948). It has been described as the aetiological agent of rainbow trout fry syndrome (RTFS) and bacterial cold water disease (BCWD) (Bernardet et al., 1996; Faruk, 2002; Starliper, 2011). Its presence on fish farms requires close surveillance and the use of antimicrobial agents to control the disease. Although many attempts have been made to develop a broad spectrum (as opposed to an autogenous) commercial vaccine against RTFS during the last 20 years, new strategies and initiatives are still needed for vaccine development, which is hindered by the prevalence of a wide range of *F. psychrophilum* strains (Gómez et al., 2014). Hence, the analysis of genetic diversity and population structure of this bacterium using molecular typing methods is essential to improve our understanding of this pathogen and, in turn, disease control at farm level.

Macrorestriction analysis by pulsed-field gel electrophoresis (PFGE) can be used to investigate the genetic variation of a bacterial population. With its high discriminatory ability and reproducibility, PFGE has been used successfully for molecular epidemiological characterisation of *F. psychrophilum* isolates in Japan (Arai et al., 2007), USA (Chen et al., 2008), Chile (Avendaño-Herrera et al., 2009), Spain (Del Cerro et al., 2010) and Finland (Sundell et al., 2013). These studies have shown that the genetic diversity of *F. psychrophilum* in these areas was associated with both geographical origin and the fish host from which the isolates were collected. Furthermore, PFGE analysis has been used to determine the source of BCWD infections (Arai et al., 2007) and to investigate the horizontal and vertical transmission of *F. psychrophilum* within and between facilities (Chen et al., 2008).

Serotyping is useful for both diagnosis and seroepidemiological studies. Three serotypes (Th, Fd, Fp^T) established by Lorenzen and Olesen (1997) and three O-antigen groups (O-1, O-2 and O-3)

* Corresponding author at: Thao Phuong Huynh Ngo, Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK.
E-mail address: tpn1@stir.ac.uk (T.P.H. Ngo).

http://dx.doi.org/10.1016/j.vetmic.2017.01.032
0378-1135/

established by Izumi and Wakabayashi (1999) are most commonly used for serotyping *F. psychrophilum* (Madsen and Dalsgaard, 2000). Some serotypes were clearly associated with the fish species from which the isolates were collected. Serotypes Th and O-3 are proposed to be broadly similar and predominant among *F. psychrophilum* strains isolated from rainbow trout in RTFS/BCWD outbreaks (Lorenzen and Olesen, 1997; Izumi and Wakabayashi, 1999; Mata et al., 2002).

In the UK, *F. psychrophilum* was first reported in diseased rainbow trout in 1992 (Santos et al., 1992). However, to date, no studies on the epidemiology of *F. psychrophilum* strains recovered in the UK have been published. Understanding the population structure of this fish pathogen is important for predicting outbreaks and setting up effective RTFS/BCWD control strategies, such as vaccination programs. The aim of this study was to describe the diversity of *F. psychrophilum* isolates from affected farms within the UK using a combination of molecular and serotyping methods.

2. Materials and methods

2.1. Bacterial isolates and growth conditions

A total of 315 *F. psychrophilum*isolates were included in this study, of which the majority of the isolates (214) were collected from diseased fish, five isolates from apparently healthy fish and the remaining 96 isolates from fish with unknown health status. From 63 sampling points, 293 *F. psychrophilum*isolates were collected from 27 sites within the UK, two of which were unknown, between 2005 – 2015 (20 sites in Scotland, six in England and one in Northern Ireland), and ten isolates were from three farms within France and Ireland (Table 1). Forty-two *F. psychrophilum*isolates collected from 29 sampling points and 12 reference strains (Table 2) in this collection were supplied by nine sources in the UK, Ireland, Denmark, Finland, France, Chile and USA. On 24 sampling occasions, multiple colonies were selected from the primary isolation plate of an infected fish sample in order to monitor the genetic variation within the *F. psychrophilum* strain population.

The presence of *F. psychrophilum* was confirmed using a nested PCR method targeting the 16S ribosomal RNA gene, as described by Toyama et al. (1994). For all the experiments, the *F. psychrophilum* isolates were routinely grown in modified Veggietone (MV) medium [veggitones GMO-free soya peptone (Oxoid, UK), 5 gL^{-1}; yeast extract (Oxoid, UK), 0.5 gL^{-1}; magnesium sulphate heptahydrate (Fisher chemicals, UK), 0.5 gL^{-1}; anhydrous calcium chloride (BHD), 0.2 gL^{-1}; dextrose (Oxoid, UK), 2 gL^{-1}; agar (solid medium; Oxoid, UK), 15 gL^{-1}; pH 7.3] at 15 °C for 72–96 h. The broth culture was shaken at 140 rpm. Stock cultures were maintained at −70 °C in tryptone–yeast extract–salts medium supplemented with glucose [FLP – tryptone (Oxoid, UK), 4.0 gL^{-1}; yeast extract, 0.4 gL^{-1}; anhydrous calcium chloride, 0.2 gL^{-1}; magnesium sulphate heptahydrate, 0.5 g L^{-1}; D(+)-glucose (Sigma, UK), 0.5 gL^{-1}; Cepeda et al., 2004] with 10% glycerol and on Protect-Multi-purpose cryobeads (Technical Service Consultants Ltd, UK).

2.2. Macrorestriction analysis by pulsed-field gel electrophoresis (PFGE)

The PFGE protocol was performed as described previously (Bartie et al., 2012) on the 315 *F. psychrophilum* isolates using restriction enzyme *Sac*I (New England BioLabs, UK) (Chen et al., 2008). The electrophoresis conditions comprised switch times of 2–6 s at 200 V at 15 °C for 37 h. Following electrophoresis, the gel was stained for 30 min in 1 μg mL^{-1} ethidium bromide (Sigma,

Table 1
Details of 303 *F. psychrophilum* isolates collected from 27 fish farm sites within the UK and three farms in Europe during 2005–2015.

Site	Year of isolation	No. of sampling times	Host source	No. of individual fish sampled	No. of isolates
Scot I	2005–2015	16	RT	35	87
Scot II	2013	2	RT	2	2
Scot III	2011–2015	5	RT	13	44
Scot IV	2013	1	RT	3	5
Scot V	2013–2015	4	RT	25	55
Scot VI	2009	1	RT	1	1
Scot VII	2007	1	RT	1	1
Scot VIII	2005	1	RT	1	1
Scot IX	2006	1	AS	1	1
Scot X	2011–2013	3	AS	3	3
Scot XI	2015	1	AS	4	4
Scot XII	2010	1	AS	1	1
Scot XIII	2005	1	AS	1	1
Scot XIV	2013	2	AS	2	2
Scot XV	2013	2	AS	8	8
Scot XVI	2014–2015	4	RT	8	14
Scot XVII	2007	1	RT	1	1
Scot XVIII	2009	1	RT	1	1
Unknown	2009–2012	2	RT	2	2
Sub-total Scotland	*2005–2015*	*50*	*RT/AS*	*113*	*234*
Eng I	2013	3	RT	8	24
Eng II	2015	1	RT	4	13
Eng III	2015	1	RT	1	2
Eng IV	2015	1	RT	1	1
Eng V	2007	1	RT	1	1
Eng VI	2007	1	RT	1	1
N Ire I	2013	2	RT	9	17
Sub-total UK	*2005–2015*	*10*	*RT/AS*	*138*	*293*
Ireland	2006	1	AS	1	1
France	Unknown – 2013	2	RT	5	9
Total		63		144	303

RT, rainbow trout; AS, Atlantic salmon.