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Vaccine-associated systemic *Rhodococcus erythropolis* infection in farmed Atlantic salmon *Salmo salar*

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ABSTRACT: In 7 instances between 2000 and 2003, clinical investigation of populations of fresh- and seawater-reared, vaccinated, Atlantic salmon *Salmo salar* suffering total losses of between 0.1 and 35% revealed infection with a Gram-positive rod-shaped bacterium. The isolations were geographically widespread, occurring in both Norway and Scotland. In all cases, a Gram-positive bacterium, subsequently identified as *Rhodococcus erythropolis*, was isolated in pure culture. Infections, although systemic, were focused within the peritoneal cavity. While initial attempts to reproduce the disease by intraperitoneal injection of unvaccinated Atlantic salmon failed, Koch's postulates were subsequently fulfilled in fish vaccinated with a commercially available oil-adjuvanted vaccine.

KEY WORDS: *Rhodococcus erythropolis* · Vaccination · Atlantic salmon · *Salmo salar* · Disease · Pathology · Peritonitis

INTRODUCTION

Bacterial infections in fish occur as the result of the interaction between host, pathogen and environment (Austin & Austin 1999), and the contribution of the farming environment towards development of disease cannot be overestimated. Thus, several bacterial fish-pathogens, e.g. *Vibrio salmonicida* and *Moritella viscosa*, have, to the best of our knowledge, never been identified in wild fish. Gram-positive bacteria represent a relatively small proportion of bacteria isolated from diseased farmed salmonids in the Northern hemisphere, and despite the increasing worldwide scrutiny of pathogens of farmed fish, serious disease due to Gram-positive bacteria (with the exception of *Renibacterium salmoninarum*) has so far been mainly limited to fish reared in warmer waters. In this paper we report on the isolation of the Gram-positive bacterium *Rhodococcus erythropolis* from several disease outbreaks involving vaccinated farmed Atlantic salmon. Evidence is presented which directly links the disease to the aquaculture process. Epidemiology, pathology, bacteriology, as well as transmission experiments are presented.

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MATERIALS AND METHODS

Fish. Moribund fish from all 7 disease outbreaks (see Table 1) were sampled for pathological and bacteriological examination.

Histopathology. Gills, heart, liver, kidney, spleen, pancreatic tissue, skeletal musculature and occasionally brain were sampled for histopathology. The samples were fixed in 4% neutral buffered formalin, embedded in paraffin wax and routinely processed. The sections were stained by haematoxylin and eosin (H&E). A selected number of slides were also stained using Gram and May Grünwald Giemsa stains.

Bacteriology. For bacteriological examination, samples from kidney and in some cases ascitic fluid, were inoculated onto blood agar (BA, 4% bovine or equine blood) and blood agar supplemented with 1.5% NaCl (BAS) followed by aerobic incubation at 22 and 15°C, respectively. Plates were observed for 7 d. Bacterial isolates were subsequently stored at –80°C.

Biochemical characterisation. Basic biochemical characterisation was performed using standard methods. Each strain was also tested using BIOLOG (Hayward) and API 20 NE (Biomerieux) kits according to the manufacturers' instructions. The ability of each strain to assimilate various carbon sources was examined by inoculating API 50 CH kits (Biomerieux) with bacteria suspended in a medium comprising 1.5 g agar, 2.0 g ammonium sulphate, 0.25 g Casamino acids (Difco) and 1 ml trace element solution.

DNA sequencing. The 16S rRNA gene of Strains 00/50/6670 and 4115 were amplified using PCR and primers FD1 and RP2 (Weisburg et al. 1991). DNA sequencing was performed using the BigDye™ terminator cycle sequencing ready reaction kit (PE Applied Biosystems) and an automatic ABI prism 377 sequencer (Perkin Elmer). Sequence analysis was performed using the sequencer program (Gene Codes) and BLAST search analysis (Altschul et al. 1997).

Phylogenetic analysis. Sequences were aligned using CLUSTAL W (Thompson et al. 1994). A neighbour-joining tree based on Kimura 2-parameter distances was calculated using PAUP* 4.0 (Swofford 2000). The tree was bootstrapped 1000 times to assess node reliability.

Enterobacterial repetitive intergenic consensus PCR (ERIC-PCR). ERIC-PCR was performed using the forward primer 5′-atg taa gct cct ggg gat tca c-3′ and reverse primer 5′-aag taa gtg act ggg gtg agc g-3′ (Ventura & Zink 2002). Each 50 µl reaction mixture contained 5 µl 10× reaction buffer (Roche), 200 µM of each deoxynucleoside triphosphates (Invitrogen), 25 pmol of each primer (Invitrogen), 2 U *Taq* polymerase (Roche) and 20 ng of the respective DNA template. Samples were denatured at 94°C for 3 min followed by 35 cycles of: 94°C × 30 s, 50°C × 60 s and 72°C × 5 min, followed by 1 cycle of 72°C for 5 min. ERIC products were separated by electrophoresis in a 1% (w/v) agarose gel at a constant voltage of 2 V cm^{-1}. PCR patterns were stained with ethidium bromide (0.5 µg ml^{-1}) and visualised under UV light at 254 nm.

Production of antisera and slide-agglutination. Antiserum against Strain 00/50/6670 was raised in a chinchilla rabbit according to the protocol of Larsen et al. (1994). Isolates representing all known outbreaks of disease were tested with this antiserum. Following incubation on BA plates at 22°C for 48 h, the test bacteria were suspended in sodium acetate-buffered saline (0.05 M NaAc, 0.1 M NaCl, 1% [v/v] formalin, pH 7.5). Equal volumes (10 µl) of bacterial suspension and antiserum were mixed and agitated. Reactions were considered positive if agglutination of bacteria occurred within 30 s.

Infection challenges. Small-scale infectious challenges on salmon pre-smolts (approximate weight 50 g) were performed at water temperatures of 4.5 and 14.5°C, respectively. The challenge bacterium (00/50/6670) used was isolated in Hordaland County (Norway) during 2000. In the first challenge, 3 groups of 5 fish were injected intra-peritoneally with 100 µl saline containing 2×10^5, 2×10^7 or 2×10^8 bacteria. In addition, 1 group of 5 fish was injected with 100 µl saline. In the second challenge, 3 fish were injected with 2×10^7 bacteria, 3 fish with 2×10^4 bacteria and 4 fish were uninjected cohabitants. In both experiments all groups were held in a single tank (100 l). The fish were fed to satiation and monitored daily for mortalities. The surviving fish were sacrificed 3 wk post-challenge and submitted to bacteriological examination, and samples were taken for histology.

Following taxonomic placement of the bacterium, a third infection trial was designed and performed. The experiment utilised 56 Atlantic salmon smolts of approximately 60 g, individually marked and held in full strength seawater (34.5‰) in a single tank of 200 l. The fish were divided into 5 groups, sedated, marked and treated as follows: Fish in Group A were injected intraperitoneally with 2×10^7 cfu *Rhodococcus erythropolis* in 0.1 ml phosphate-buffered saline (PBS), Group B received 2 separate injections of 2×10^7 cfu *R. erythropolis* in 0.1 ml PBS + 0.1 ml vaccine (multivalent, oil-adjuvanted), Group C received a single injection of 2×10^7 cfu *R. erythropolis* in 0.1 ml PBS + 0.1 ml vaccine. Control Groups D and E received 0.1 ml vaccine and 0.1 ml PBS, respectively. The vaccine used was of the same type, but of a different batch, to that used prior to 2 of the field outbreaks, and was kindly provided by the manufacturer. The vaccination/challenge was initiated 1 wk following exposure of the fish to full

strength seawater. The water temperature was raised on the day of the challenge from 9 to 12°C, and was maintained at that level for the remainder of the trial.

Fifteen fish, scattered among Groups B to E, did not recover from the initial sedation, and the experimental population was thereby reduced to 41 individuals (11 fish in Group A, 5 in Group B, 9 in Group C, 9 in Group D and 7 in Group E). Dead fish were removed daily for the duration of the experiment, which lasted for 45 d. Post-mortem examination and routine bacteriology from kidney and occasionally ascitic fluid was performed on all fish that died and on surviving fish sacrificed at the end of the experiment.

RESULTS

Epidemiology

The cases reported comprised 2 cases in Scotland and 5 cases in Norway, in farmed Atlantic salmon (Table 1). Mortality levels in the Scottish farms involved were lower than those in the Norwegian farms, where reported cumulative mortalities ranged between 1.3 and 35% in affected populations. In Norway the first detection was in post-smolts 1 mo following sea transfer (Olsen et al. 2001), and all other cases were diagnosed prior to sea transfer. One of the 5 Norwegian cases occurred (unforeseen) in fish under experimental conditions. Onset of infection occurred at high as well as at low water temperatures.

Affected farms in both countries were widely separated geographically. All cases occurred in fish intraperitoneally vaccinated in the freshwater phase with either monovalent (Scotland) or multivalent (Norway) oil-adjuvanted vaccines (Table 1), produced by more than 1 vaccine manufacturer.

Gross pathology

Moribund fish suffered from scale loss and, occasionally, cutaneous haemorrhage of the abdomen and the bases of the pectoral and abdominal fins were observed; some fish showed distention of the abdomen (Fig. 1). Bilateral exophthalmia was reported in the Scottish cases. Internal findings, including splenomegaly, petechiation of parietal peritoneum and serous to serohaemorrhagic ascites, were consistent with a systemic infection. A typical finding was severe peritonitis, visible as a whitish, loose to compact pseudo-membrane, in some cases containing small fluid- or 'pus'-filled cavities, covering internal organs. Vaccine-related peritoneal adhesions were common in all fish and the stomach had a seromucoid content.

Histopathology

Histopathology revealed the presence of Gram-positive, rod-shaped bacteria (Figs. 3 to 6), with the bacteria being especially numerous in association with subacute to chronic vaccine-related peritonitis (Figs. 3, 5 & 6). Aggregates of bacteria and accompanying inflammatory reaction were also observed subepicardially, and occasionally in skeletal musculature. Bacterial colonies were found intravascularly in different organs (Fig. 4) and as intra-luminal thrombi in the spongy myocardium. Diseased fish usually displayed leucocytosis.

Table 1. *Salmo salar.* Known outbreaks of infection with *Rhodococcus erythropolis* in farmed Atlantic salmon. Age/wt: age of fish and weight at time of diagnosis; Diagnosis time: time from vaccination to diagnosis of disease; Mortal.: cumulative mortality; *T*: temperature at time of vaccination; N: Norway; S: Scotland; fw: freshwater; br: brackish water; sw: seawater; na: no isolate available; nd: no data

Outbreak	Bacterial isolate	Age/wt	Water	Diagnosis		Mortal. (%)	*T* (°C)	Vaccine
				Date	Time			
N-1	00/50/6670	Smolt/150 g	sw	Nov 2000	3 mo	23[a]	nd	A[c]
N-2	02/50/2284	Parr/50 g	fw/br	Aug 2002	14 d	11	18–20	A
N-3	03/50/822	Smolt/70 g	fw	Feb 2003	3 mo	1.3	1.8–3	B[d]
N-4	03/09/159	Smolt/70 g	fw	Dec 2002	17 d	20–35[b]	12	nd
N-5	03/40/5697	Smolt/80 g	fw	Mar 2003	4.5 mo	3	5	B
S-1	na	Adult/1 kg	sw	Jul 2000	8 mo	<0.5	6	C[e]
S-2	4115	Post-smolt/300 g	sw	Sep 2000	10 mo	<0.1	6	C

[a]Also other disease problems, antibiotic treatment in February 2001
[b]Mortality within 2 test groups of 50 individuals each in tank with total population of 700 fish
[c]Multivalent, oil-adjuvanted, 5 bacterial antigens
[d]Multivalent, oil-adjuvanted, 5 bacterial antigens and 1 virus antigen
[e]Monovalent, oil-adjuvanted, 1 bacterial antigen

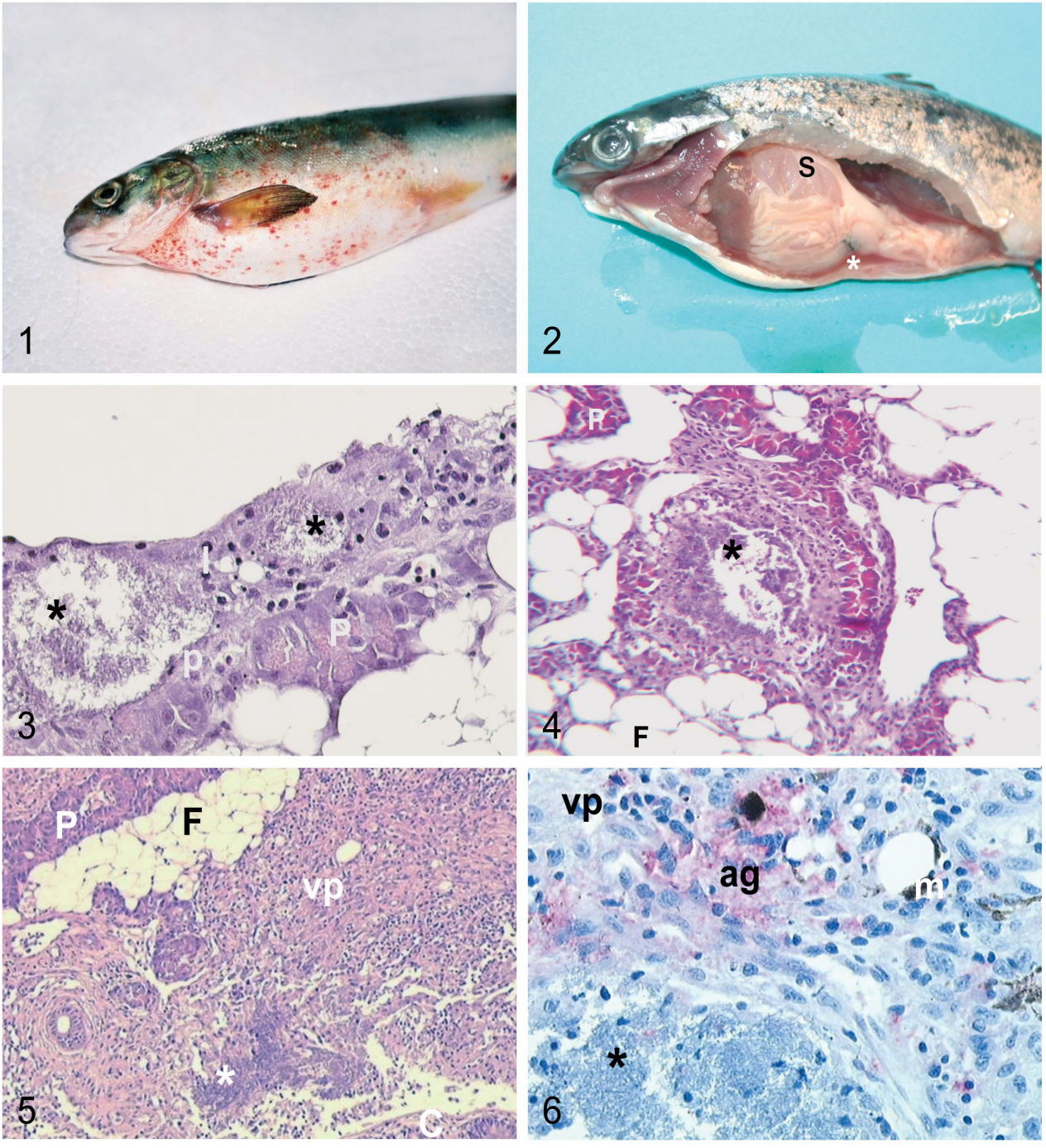

Fig. 1. *Salmo salar*. Parr naturally infected by *Rhodococcus erythropolis*, showing extensive haemorrhaging of skin and distended abdomen. Fig. 2. *Salmo salar*. Smolt from Infection Challenge 3, dead 9 d after intraperitoneal injection with both *Rhodococcus erythropolis* and oil-adjuvanted vaccine. Stomach is very distended (s), with seromucoid content. Serohaemorrhagic ascites present (*). Fig. 3. *Salmo salar*. Smolt naturally infected by *Rhodococcus erythropolis*. Peritonitis with numerous bacteria (*) and leucocytes (l). p: peritoneal lining; P: normal exocrine pancreas. Haematoxylin and eosin (H&E) staining. Fig. 4. *Salmo salar*. Smolt naturally infected by *Rhodococcus erythropolis*. Bacteria within large vessel (*); F: perivisceral fatty tissue; P: exocrine pancreas. H&E. Fig. 5. *Salmo salar*. Smolt naturally infected by *Rhodococcus erythropolis*. Chronic, vaccine-induced, granulomatous peritonitis (vp). Pockets with aggregates of *Rhodococcus erythropolis* (*). F: perivisceral fatty tissue; P: exocrine pancreas; C: pyloric caeca. H&E. Fig. 6. *Salmo salar*. Smolt naturally infected by *Rhodococcus erythropolis*. Chronic vaccine-induced peritonitis (vp) with infiltration of melanomacrophages (m). ag: *Aeromonas salmonicida* ss *salmonicida* antigen, a vaccine component, is stained red by immunohistochemistry. Large aggregate of *R. erythropolis* is visible adjacent to peritoneal reaction (*). H&E. Original magnifications: (3) ×200, (4) ×200, (5) ×40, (6) ×400

Bacteriology

Phenotypic analysis

The bacteria described were isolated in pure culture or as the dominant colony type in mixed cultures from all fish examined. All isolates produced round, shiny, convex, beige and non-haemolytic colonies on BA. All isolates were strictly aerobic, Gram-positive rods, and were cytochrome oxidase negative and catalase positive. Growth rate was reduced with the addition of 1.5% NaCl to the BA medium. The bacteria grew better at 30°C than at 15°C, but there was no growth at 4 or 37°C. Differences occurred in carbohydrate utilisation, as shown in Tables 2 & 3.

Slide agglutination

Positive agglutination was achieved with the isolate used for antiserum production (00/50/6670) and in 1 other isolate (03/40/5697). Weak agglutination was observed with the type strain (NCIMB 11148). The remaining isolates gave negative results.

Genotypic analysis

Nearly complete 16S rDNA sequences were obtained from 1 Norwegian and 1 Scottish isolate and these were submitted to Genbank under Accession Nos. AY147846 (00/50/6670) and AJ505559 (4115), respectively. Alignment of these sequences revealed significant homology (99.8%) between the 2 sequences. BLAST searching revealed high degrees of similarity to the genus *Rhodococcus*, with identity levels of 100 and 99.9% for Norwegian and Scottish isolates, respectively, with *R. erythropolis* ATCC13260. The relationships between the strains are presented as a phylogenetic tree in Fig. 7.

ERIC-PCR

PCR amplification using ERIC I and ERIC II primers produced a distinctive fingerprint for each strain. While the 2 reference strains produced quite different profiles, common bands were identified in the Scottish isolate, several Norwegian isolates and *Rhodococcus erythropolis* Type Strain NCIMB 11148. No 2 isolates produced identical profiles (Fig. 8).

Infectivity trial

Infection Challenges 1 and 2

Fish began to feed on the day following injection of the bacteria; they appeared healthy and maintained good appetite throughout the remainder of the experimental period. No mortalities were recorded and no bacteria were isolated from the fish sacrificed at the end of the trials. No pathological changes were found following histological examination of formalin-fixed tissues.

Infection Challenge 3

With the exception of 2 individuals from the group receiving vaccine alone, mortality was confined to the 2 groups challenged with both bacteria and vaccine (Fig. 9). Heavy growth of the bacterium was achieved from kidney tissue in all dead fish and in the ascitic

Table 2. Isolate differences in carbohydrate utilisation using AP20NE and AP50CH. All isolates were positive for utilisation of glycerol, glucose, inositol, mannitol, sorbitol, N acetyl glucosamine, sucrose, trehalose, D-arabitol, adipate, malate, phenylacetate and gluconate; all isolates were negative for utilisation of D-arabinose, L-arabinose, D-xylose, L-xylose, β-methyl xyloside, galactose, D-mannose, L-sorbose, rhamnose, dulcitol, α-methyl-D-mannoside, α-methyl-D-glucoside, D-turanose, amygdalin, caprate, esculin, salicin, cellobiose, maltose, lactose, melibiose, inulin, melizitose, D-raffinose, starch, glycogen, β-gentibiose, D-lyxose, D-tagatose, D-fucose, L-fucose and 2 keto-gluconate

Substrate	NCIMB 11148	ATCC 13260	00/50/6670	4115	03/50/822	03/40/5697	03/09/159	02/50/2284a	02/50/2284b
Erythritol	–	–	–	–	+	+	–	–	+
Ribose	–	+	+	+	+	+	+	+	+
Adonitol	–	–	–	–	+	+	–	–	+
Arbutin	–	–	+	–	–	–	–	–	–
Xylitol	+	+	–	+	–	–	–	–	+
L-arabitol	–	–	–	–	+	+	–	+	+
5 keto-gluconate	+	–	–	–	+	–	+	–	–
Citrate	+	+	+	+	–	+	+	+	+

Table 3. Isolate differences in carbohydrate utilisation using BIOLOG. All isolates were positive for utilisation of Tween 40, Tween 80, D-arabitol, D-fructose, D-mannitol, D-sorbitol, monomethyl succinate, acetic acid, D-gluconic acid, β-hydroxybutyric acid, propionic acid, quinic acid, sebacic acid, succinic acid, bromosuccinic acid, D-alanine, L-alanine, L-asparagine, L-leucine, L-phenylalanine, urocanic acid, putrescine, 2-aminoethanol and glycerol; all isolates were negative for utilisation of cyclodextrin, N-acetyl-D-galactosamine, L-arabinose, L-fucose, D-galactose, gentobiose, α-lactose, α-D-lactose-lactulose, D-melibiose, β-methyl-D-glucoside, psicose, saccharic acid, succinamic acid, glucuronamide, L-glutamic acid, L-histidine, hydroxy-L-proline, L-ornithine, D-serine, D,L-carnitine, D-raffinose, L-rhamnose, sucrose, D-trehalose, turanose, xylitol, cis-aconitic acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, γ-hydroxybutiric acid, p-hydroxyphenylacetic acid, itaconic acid, malonic acid, inosine, uridine, thymidine, phenylethylamine, D,L,-α-glycerophosphate and glucose-1-phosphate

Test	NCIMB 11148	ATCC 13260	00/50/6670	4115	03/50/822	03/40/5697	03/09/159	02/50/2284a	02/50/2284b
Dextrin	–	–	+	–	–	–	–	–	–
Glycogen	–	–	+	–	–	–	–	–	–
N-acetyl-D-glucosamine	+	+	+	–	+	+	+	+	+
Adonitol	–	+	–	–	+	–	–	–	–
Cellobiose	–	–	+	–	–	–	–	–	–
l-erythritol	–	+	–	–	+	+	–	–	–
α-D-glucose	–	–	+	–	+	–	–	–	–
m-inositol	+	+	+	–	+	+	+	+	+
Maltose	–	–	+	–	–	–	–	–	–
D-mannose	–	–	+	–	–	–	–	–	–
Methyl pyruvate	–	–	–	–	+	+	+	–	–
Citric acid	+	+	+	–	–	–	–	+	+
α-hydroxybutyric acid	+	+	+	–	+	+	+	+	–
α-keto glucaric acid	–	–	–	–	–	+	–	–	–
α-keto butyric acid	+	+	+	–	+	+	+	+	–
α-keto valeric acid	+	+	+	–	+	+	+	+	+
D,Ll-lactic acid	+	–	+	–	+	+	+	–	+
Alaninamide	–	–	–	–	+	–	–	–	–
L-alanylglycine	+	+	–	–	+	+	+	+	–
L-aspartic acid	–	–	+	–	–	+	–	–	–
Glycyl-L-aspartic acid	–	–	+	–	–	–	–	–	–
Glycyl-L-glutamic acid	–	–	+	–	–	+	–	–	–
L-proline	–	+	+	+	+	+	+	+	–
L-pyroglutamic acid	–	+	–	–	–	–	–	+	–
L-serine	–	–	+	–	+	+	+	–	–
L-threonine	–	–	+	–	+	+	+	–	–
γ-amino butyric acid	–	+	+	–	+	+	+	–	–
2,3 butanediol	–	+	+	+	+	+	+	+	+
Glucose-6 phosphate	–	–	–	+	–	–	–	–	–

fluid of the 5 fish for which this fluid was examined. Although a distended abdomen was observed only in some individuals, all dead fish displayed gross pathological changes, including hyperaemia of the injection site, fin bases and gut. Internal findings for all individuals were a varying amount of seromucoid content in stomach, serous or serohaemorrhagic ascitic fluid and whitish, loose material covering the abdominal organs; 2 individuals displayed particularly extensive pathological changes, as shown in Fig. 2. Sparse to moderate growth of *Rhodococcus erythropolis* was recorded for the 2 fish which died in the group injected with vaccine alone.

DISCUSSION

Bacterial strains such as those found during the present study are difficult to speciate using the standard phenotypical techniques available in most aquatic microbiology laboratories. *Rhodococcus erythropolis* is rather poorly phenotypically described in the literature. As can be seen in Tables 2 & 3, the phenotypic profiles of type and reference strains provides evidence that *R. erythropolis* as a species is somewhat heterogeneous. Although the isolates under study also revealed several variable traits, many more traits were common to all, and we conclude therefore that Norwe-

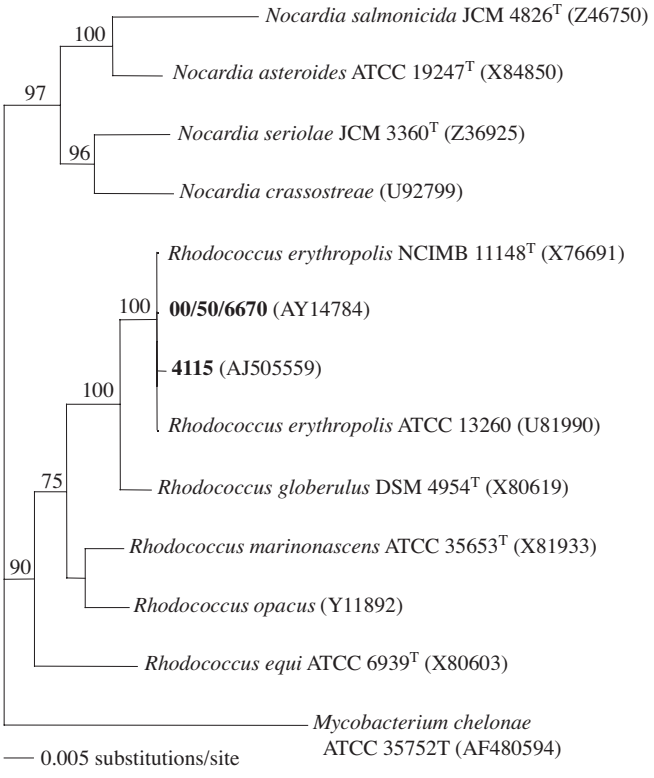

Fig. 7. Neighbour-joining phylogenetic tree constructed using 16S rDNA sequences comparing 2 isolates from present study (NVI 00/50/6670 and 4115) with reference isolates. *Mycobacterium chelonae* included as outgroup. Culture collection references are given where applicable. ATCC: American Type Culture Collection; NCIMB: National Collection of Industrial and Marine Bacteria; JCM: Japan Collection of Microorganisms. GenBank accession numbers in parentheses

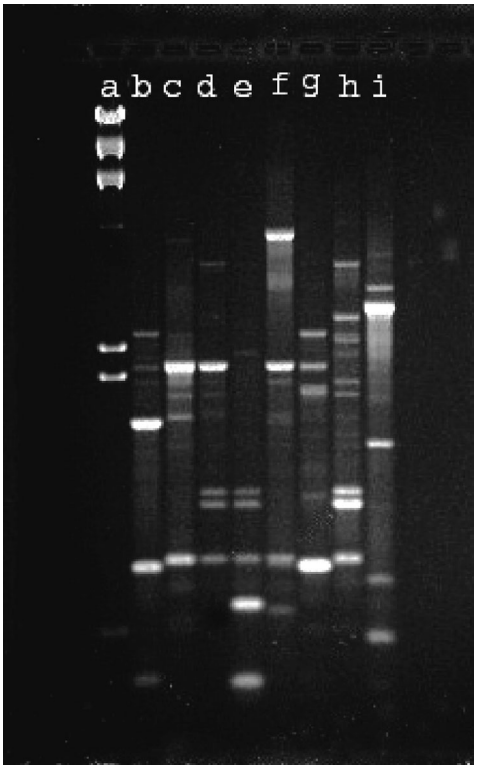

Fig. 8. *Rhodococcus erythropolis*. ERIC-PCR of strains isolated from diseased salmon *Salmo salar*. Lane a: molecular weight marker Lamda *Hin*dIII; b: ATCC 13260; c: NCIMB 11148^T; d: 4115; e: 02/05/822; f: 03/40/5697; g: 00/50/6670; h: 02/50/2284; i: 03/09/159

gian Isolate 00/50/6670 and Scottish Isolate 4115, for which ribosomal sequence and ERIC-PCR data are available, belong to the species *R. erythropolis* and that the remaining Norwegian isolates with basically similar phenotypical profiles in all probability also belong to this species.

The isolation of the bacterium from all diseased individuals in all cases and the consistent finding of pathological changes associated with large numbers of Gram-positive rods morphologically similar to *Rhodococcus erythropolis* (as revealed by histopathological examination) strongly indicates an association between pathology and the isolated bacterium.

While we were unable to establish disease by challenge with *Rhodococcus erythropolis* alone, mortalities reached 100% in fish simultaneously vaccinated with oil-adjuvanted vaccines. *Rhodococcus* spp. are frequently used in industrial bioremediation of hydrocarbons, including mineral oils (Castorena et al. 2002), and *R. erythropolis* has been shown to be a particularily active species (Zviagintseva et al. 2001). It may be speculated that the oil component of oil-adjuvanted vaccines provides a source of nutrition and may also provide a degree of physical protection from the immune system of the fish. A strong association between intraperitoneal immunisation with an oil-based vaccine and infection with *R. erythropolis* is also supported by the consistent and main histopathological findings in field cases showing large numbers of bacteria and leucocytes together with vaccine-induced peritonitis. Also, 2 field cases were diagnosed within 2 to 3 wk of vaccination. In 1 of these 2 cases the association between vaccination and infection is strong, as the nature of the outbreak (unplanned infection in a stock of experimental fish) allowed close monitoring of the course of disease. There was, however, no consistency between the time of vaccination and the detection of the infection, as in 5 cases no disease was seen until some months later, even after sea transfer (3 instances). These fish may have been infected at a later stage, but may also have been long-term asymptomatic carriers. As the preferred growth temperature for our isolates seems to be in the warmer range, i.e. above 22°C, and growth is inhibited by the addition of NaCl, we also believe that exposure of these fish to the

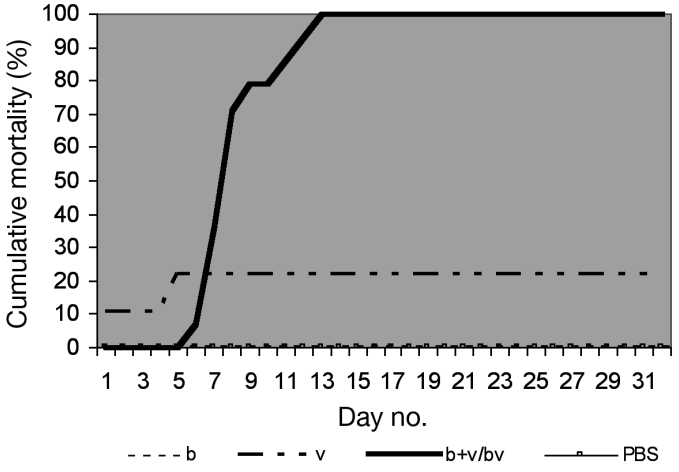

Fig. 9. *Salmo salar*. Infection challenge with intraperitoneally injected *Rhodococcus erythropolis* alone and with oil-adjuvanted vaccine, showing cumulative percentage mortality in challenge groups during observation period. b: bacteria; v: vaccine; b+v: bacteria and vaccine in 2 separate injections; bv: bacteria and vaccine in same injection; PBS: phosphate-buffered saline

bacterium in the freshwater phase is most likely. Evidence for transmission of the bacteria from infected fish to non-infected vaccinated fish exists. In both our third experimental infectious challenge and during a natural outbreak within a population of experimental fish, transmission to non-infected groups was identified.

The natural source of the infection is not known. *Rhodococcus erythropolis* is described from terrestrial (Saadoun 2002), marine (Heald et al. 2001) and freshwater (van der Wef et al. 1999) environments. We believe that the rather limited number of outbreaks seen so far indicates that the bacterium is not endemic in the salmon populations. The fish may have become infected as the result of a suboptimal hygienic environment at the time of vaccination, i.e. soil-contaminated water or contaminated needles. Questionable vaccination standards were reported for some fish groups in this study (epidemiological data not shown), but infection also occurred in fish held in a strictly controlled environment. ERIC-PCR analysis supported by serological studies indicates that while a degree of relatedness exists between several of the strains, they are genetically quite heterogeneous. Although the possibility exists that the bacteria may have been introduced to the fish via the vaccine itself, the evidence suggests that no common source of infection exists and that contamination of vaccine components during manufacture is therefore unlikely. Indeed, one would probably also have expected larger outbreaks if batches of contaminated vaccine had been the source of infection.

In farmed salmonids, 2 cases of infection by *Rhodococcus* spp. have previously been reported. Backman et al. (1990) describe an outbreak of panophthalmitis in farmed chinook salmon with the presence of a *Rhodococcus* sp. alone or together with a *Mycobacterium* sp. Chronic granulomatous nephritis in juvenile Atlantic salmon and the isolation of a *Rhodococcus* sp. was reported by Claveau (1991). Although the *Rhodococcus* spp. isolated in these cases clearly differed phenotypically, the description given by Claveau (1991) may suggest a relationship between that isolate and the *R. erythropolis* isolates presented here. In conclusion, although further studies are necessary to elucidate the pathogenesis of this vaccine-related infection, it adds to the growing list of production-related 'diseases' previously described in vaccinated Atlantic salmon (Poppe & Breck 1997, Koppang et al. 2003, 2004, 2005) and indicates the potential for further refinement of both vaccines and vaccination procedures.

We believe this is the first description of infection in fish by *Rhodococcus erythropolis*. It is also, to our knowledge, the first demonstration that exposure to oil-adjuvanted vaccine may predispose the vaccinated fish to infection with an otherwise harmless bacterium.

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