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NOTE

Pseudomonas anguilliseptica isolated from Baltic herring *Clupea harengus membras* with eye lesions

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ABSTRACT: Strains of *Pseudomonas anguilliseptica* were isolated from Baltic herring *Clupea harengus membras* L. caught on the southwest coast of Finland and showing hemorrhages in the eyes. When compared to the type strain of *P. anguilliseptica* (NCMB 1949), the strains examined showed only minor biochemical differences. The strains isolated from Baltic herring were of low pathogenicity to rainbow trout *Oncorhynchus mykiss*. This is the first isolation of *P. anguilliseptica* from wild fish, and the possibility of the wild fish serving as a vector of this pathogen for farmed salmonids is discussed.

KEY WORDS: *Pseudomonas anguilliseptica* · *Clupea harengus membras* · Baltic Sea · Eye lesion

Pseudomonas anguilliseptica was originally isolated from pond-cultured Japanese eels *Anguilla japonica* suffering from 'Sekiten-byo', i.e. red spot disease (Wakabayashi & Egusa 1972). Later on, the infection occurred in eels farmed in Taiwan (Kuo & Kou 1978), Scotland (Nakai & Muroga 1982, Stewart et al. 1983), Denmark (Mellergaard & Dalsgaard 1986), and France (Michel et al. 1992). The bacteria were also isolated from cultured black sea bream *Acanthopagrus schlegeli* (Nakajima et al. 1983) and ayu *Plecoglossus altivelis* (Nakai et al. 1985).

In Finland, *Pseudomonas anguilliseptica* caused disease outbreaks in farmed salmonid fish in 1986 (Wiklund & Dalsgaard 1987, Wiklund & Bylund 1990). An increasing number of Finnish fish farms have been suffering from the disease in recent years (unpubl. results). To our knowledge, however, there are no previous reports concerning isolation of *P. anguilliseptica* from wild fish.

This paper describes the characteristics of *Pseudomonas anguilliseptica* isolated from Baltic herring *Clupea harengus membras* L. with eye lesions. The fish were caught on the southwest coast of Finland.

Material and methods. ***Fish sampling:*** Baltic herring to be examined were caught with standing gill nets and bow nets in May and June 1990 and 1991, near the town of Pori, in southwestern Finland. The fish were transported to our laboratory for bacteriological examination. A total of 21 fish were examined.

Bacteriological sampling: Bacteriological samples were taken from the eyes, liver, spleen, and kidney of the fish. The primary isolations were made on tryptic soy agar (Difco Laboratories, Detroit, MI, USA) supplemented with 5 % bovine blood. The final NaCl concentration in the medium was 1.5 %. The inoculated agar plates were incubated for 7 d at 20 °C before being discarded.

The type strain of *Pseudomonas anguilliseptica*, NCMB 1949, was included as a reference strain. Four strains of *P. anguilliseptica* recently isolated from wild and cultivated Atlantic salmon *Salmo salar* and sea trout *Salmo trutta* m. *trutta*, obtained from the same sampling area as the herring, were tested concurrently.

Biochemical tests: The biochemical characteristics of the isolates were examined according to the methods described by Cowan (1974), MacFaddin (1983), and Wiklund & Bylund (1990). The O/F test was performed on a medium modified for marine bacteria (MOF) (Leifson 1963). The ability of the strains to produce acid from carbohydrates was tested using the following sugars: arabinose, fructose, lactose, mannose, salicin, sucrose, and trehalose.

The cytophaga agar was made according to Anacker & Ordal (1959) except that the amount of agar was increased to 15 g l^{-1} to facilitate streaking. The sensitivity of the isolates to different chemotherapeutants was

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Table 1. *Pseudomonas anguilliseptica*. Isolation rate from different organs of Baltic herring

Organ	No. infected / no. examined	%
Eye	3/9	33
Kidney	6/20	30
Liver	0/13	0
Spleen	0/10	0
Eye, kidney, liver or spleen	9/21	43

tested using the disc diffusion technique (Neo-sensitabs, Rosco, Taastrup, Denmark) on Mueller-Hinton agar. The media used for the biochemical tests were manufactured by Difco Laboratories. Unless otherwise stated, tests were performed at 20°C.

Serology: Slide agglutination tests were performed on all strains (including the reference strain) using a diluted (1:10) rabbit serum containing antibodies against the O-antigen from the *Pseudomonas anguilliseptica* strain 12-48. This strain was originally isolated from farmed rainbow trout *Oncorhynchus mykiss* in Finland.

Challenge tests: The pathogenicity of 1 isolate of *Pseudomonas anguilliseptica* (12-93, isolated from Baltic herring) was assessed by intraperitoneal injection (i.p.) of rainbow trout with 0.1 ml of twice washed bacteria suspended in sterile 0.9 % NaCl. The strain used was passed twice through rainbow trout before the challenge. Four groups of fish weighing 38 to 50 g were injected with 8.0×10^{10}, 8.0×10^{8}, and 8.0×10^{6} colony-forming units ml^{-1} (CFU ml^{-1}) of strain 12-93, and with saline, respectively. Each group consisted of 9 or 10 fish. The fish were kept in plastic tanks with filtered, aerated, flow-through tap water (temperature 15°C) and fed a commercial pellet feed. Mortality was recorded for 2 wk after injection. Kidney and spleen samples from all fish, dead or surviving, were examined bacteriologically.

Results. ***Clinical signs:*** The major external sign of disease in affected herring was hemorrhages in the eyes. The cornea was punctured in some of the fish. Other clinical signs included hemorrhages in the fins and on the head. Accumulation of blood-stained ascites was seen in several of the fish examined.

Bacteriology: A total of 21 herring with eye lesions were examined for bacterial infection in the eyes and/or the internal organs (Table 1). Pure cultures of a slow-growing, non-pigmented bacterium occurred on the agar plates inoculated with tissues from 9 (43 %) fish, i.e. from 33 % of the eyes, and from 30 % of the kidneys examined. No bacterial growth occurred from the liver or spleen of the examined fish.

The isolated bacteria were Gram negative, motile, cytochrome oxidase positive rods that showed a negative reaction in Leifson's modified O/F medium. The strains tested did not grow on *Pseudomonas* isolation agar. The biochemical reactions of the isolates from herring and salmonids corresponded, with minor deviations, with those of the *Pseudomonas anguilliseptica* type strain tested (Table 2) except that the present isolates did not liquefy gelatin nor did they hydrolyze Tween 80. Isolates from herring and salmonids were sensitive to cephalosporins but the type strain was resistant. All strains were agglutinated with rabbit antiserum to strain 12-48. On the basis of the results obtained, the strains isolated from herring were identified as *Pseudomonas anguilliseptica*.

Challenge tests: In the tests performed with strain 12-93 isolated from herring, 5 out of 9 rainbow trout injected with the highest dose of the bacterium died (Table 3). Mortality occurred within 8 d of injection without any gross clinical signs. *Pseudomonas anguilliseptica* was reisolated from the kidney and spleen of 4 dead fish. A *Pseudomonas* sp. was isolated from the remaining dead fish. No mortality occurred in the 2 groups challenged with the lower doses of the bacterium. The injected bacteria were reisolated from 1 surviving fish in each of the 3 groups tested.

Discussion. The present results showed that the isolates from Baltic herring were identical to *Pseudomonas anguilliseptica*, which had caused serious disease outbreaks in farmed salmonid fish in Finland (Wiklund & Bylund 1990).

In recent years *Pseudomonas anguilliseptica* had frequently been isolated in our laboratory during disease outbreaks in rainbow trout farms. The mode of transmission of the pathogen from farm to farm is still unknown. As herring frequently occur in the vicinity of the rainbow trout net pens on the Finnish coast, these fish might act as vectors for transfer of *P. anguilliseptica*.

In the biochemical tests no differences between the isolates from salmon and herring were detected. All strains tested differed from the type strain regarding degradation of gelatin and Tween 80, and susceptibility to cephalosporins. When compared with strains previously isolated in Finland (Wiklund & Bylund 1990), our herring isolates differed only in the gelatinase test. However, according to Michel et al. (1992) gelatinase activity is easily lost and cannot be considered a reliable marker of *Pseudomonas anguilliseptica*.

The strain isolated from Baltic herring was of low pathogenicity to rainbow trout in laboratory tests. The recovery of *Pseudomonas anguilliseptica* from rainbow trout surviving the challenge suggests, however, that the bacterium can establish a carrier state in these fish. Previous reports indicated that the pathogenicity of

Table 2. *Pseudomonas anguilliseptica*. Biochemical characteristics of bacteria isolated from Baltic herring and salmonids compared with those of the type strain NCMB 1949. +: positive reaction; –: negative reaction; S: sensitive; R: resistant; (n/n): n out of n tested show positive reaction

	Isolates from Baltic herring (n = 10)	Isolates from salmonids (n = 4)	Type strain NCMB 1949
Gram stain	–	–	–
Morphology	rod	rod	rod
Motility	+	+	+
Cytochrome oxidase	+	+	+
Catalase	+	+	+
Modified O/F test	–	–	–
Arginine dehydrolase	–	–	–
Lysine decarboxylase	–	–	–
Ornithine decarboxylase	–	–	–
Growth at:			
5 °C	+ (8/10)	+	+
30 °C	+ (9/10)	+ (2/4)	+
37 °C	–	–	–
Growth in:			
0 % NaCl	+	+	+
3 % NaCl	+	+	+
4 % NaCl	–	–	–
Growth on:			
Cytophaga agar	+	+	+
MacConkey agar	+	+	+
Pseudomonas isolation agar	–	–	–
TCBS agar	–	–	–
Production of:			
β-galactosidase (ONPG)	–	–	–
Hydrogen sulphide (SIM)	–	–	–
Indole (Tryptone broth)	–	–	–
Pigment (Kings A and B)	–	–	–
Acid from carbohydrates	–	–	–
Citrate utilization	+ (4/10)	+ (3/4)	+
Nitrate reduction	–	–	–
Degradation of:			
Blood (haemolysis)	–	–	–
Casein	+ (5/10)	+ (3/4)	+
DNA	–	–	–
Esculin	–	–	–
Gelatin	–	–	+
Starch	–	–	–
Tween 80	–	–	+
Susceptibility to:			
Ampicillin (33 µg)	S	S	S
Cephalothin (66 µg)	S	S	R
Oxolinic acid (10 µg)	S	S	S
Oxytetracycline (80 µg)	S	S	S
Trimethoprim + sulfa (5.2 + 240 µg)	S	S	S
0/129 (150 µg)	R	R	R

P. anguilliseptica varied between different species. Muroga et al. (1975) reported that *P. anguilliseptica* isolated from eels was highly pathogenic to Japanese eel, ayu, loach *Misgurnus anguillicaudatus*, and bluegill sunfish *Lepomis macrochirus*. On the other hand, the pathogenicity to carp *Cyprinus carpio*, crucian carp *Carassius carassius*, and goldfish *Carassius auratus* was low. Uno (1976) found none of the salmonids tested to be sensitive to *P. anguilliseptica*.

An eye disease among Baltic herring on the southwest coast of Finland was first observed 15 yr ago (Järvinen 1983), but the etiology of the disease was not

Table 3. *Pseudomonas anguilliseptica* (*P.a.*) infecting *Oncorhynchus mykiss*. Challenge test on rainbow trout (weight 38 to 50 g) with bacterium (strain12-93) isolated from Baltic herring

Group	Injected dose (CFU fish^{-1})	No. of fish dead / no. of fish tested	No. of dead fish yielding *P.a.* / no. of dead fish	No. of surviving fish yielding *P.a.* / no. of surviving fish
I	8.0×10^9	5/9	4/5	1/4
II	8.0×10^7	0/10	0/0	1/10
III	8.0×10^5	0/10	0/0	1/10
IV	(physiol. saline)	0/10	0/0	0/10

firmly established. During investigations conducted in 1982, Gram negative rods were observed in histological samples from the eyes of herring with eye lesions. Bacteriological samples were taken only from the blood and kidney of the fish but no bacteria were isolated (Bylund 1983).

In previous investigations of cod *Gadus morhua* with eye diseases, Bergman (1912) isolated *Vibrio anguillarum*-like bacteria. *Aeromonas* sp., *Pseudomonas* sp., *Vibrio* sp., flavobacteria, acid-fast bacteria (Dukes 1975), and a *Corynebacterium* sp. (Baya et al. 1992) have also been isolated from fish suffering from exophthalmos. However, exophthalmos is a general disease sign associated with a number of bacterial infections.

The role of *Pseudomonas anguilliseptica* as the etiological agent of the eye lesions in the Baltic herring remains to be investigated. The fact that the pathogen was isolated from less than 50 % of the examined fish in the present investigation might indicate that the bacterium represented a secondary infection.

Today efforts are being initiated to increase the use of Baltic herring as feed for farmed salmonids in Finland. This might be a potential hazard for the exposed salmonids. Even if *Pseudomonas anguilliseptica* is not highly pathogenic to rainbow trout under laboratory conditions, stress factors could promote serious disease outbreaks in farmed salmonid populations that carry the pathogen. Previous outbreaks (Wiklund & Bylund 1990) support this suggestion.

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LITERATURE CITED

Anacker, R. L., Ordal, E. J. (1959). Studies on the myxobacterium *Chondrococcus columnaris*. I. Serological typing. J. Bacteriol. 78: 25–32

Baya, A. M., Lupiani, B., Bandín, I., Hetrick, F. M., Figueras, A., Carnahan, A., May, E. M., Toranzo, A. E. (1992). Phenotypic and pathobiological properties of *Corynebacterium aquaticum* isolated from diseased striped bass. Dis. aquat. Org. 14: 115–126

Bergman, A. M. (1912). Eine ansteckende Augenkrankheit, Keratomalacie, bei Dorschen an der Südküste Schwedens. Centralbl. f. Bakteriologie Abt. I. Orig. Bd. 62: 200–212

Bylund, G. (1983). Porin edustan merialueen silakan terveydentila sekä silmävikaisten kalojen taudinkuva. (The health of Baltic herring and the symptoms of eye disease in fish in Pori coastal waters.) Meri 12: 203–212

Cowan, S. T. (1974). Cowan & Steel's manual for the identification of medical bacteria, 2nd edn. Cambridge University Press, Cambridge

Dukes, T. W. (1975). Ophthalmic pathology of fishes. In: Ribelin, W. E., Migaki, G. (eds.) The pathology of fishes. The University of Wisconsin Press, Madison, p. 383–398

Järvinen, A. (1983). Silmävikaisten silakoiden esiintymisestä selkämerellä. (Baltic herring with eye disease in the Bothnian Sea.) Meri 12: 213–224

Kuo, S.-C., Kou, G.-H. (1978). *Pseudomonas anguilliseptica* isolated from red spot disease of pond-cultured eel, *Anguilla japonica*. Rep. Inst. Fish. Biol., Min. Econ. Aff., Nat. Taiwan Univ. 3: 19–23

Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. J. Bacteriol. 85: 1183–1184

MacFaddin, J. F. (1983). Biochemical tests for identification of medical bacteria, 2nd edn. Williams & Wilkins, Baltimore

Mellergaard, S., Dalsgaard, I. (1986). Håndbog i Ålesygdomme. Danmarks fiskeri- og havundersøgelser, Rapport no. 293: 33–34

Michel, C., Bernardet, J.-F., Dinand, D. (1992). Phenotypic and genotypic studies of *Pseudomonas anguilliseptica* strains isolated from farmed European eels (*Anguilla anguilla*) in France. Gyobyo Kenkyu 27: 229–232

Muroga, K., Jo, Y., Sawada, T. (1975). Studies on red spot disease of pond-cultured eels. II. Pathogenicity of the causative bacterium, *Pseudomonas anguilliseptica*. Fish Pathol. 9: 107–114

Nakai, T., Hanada, H., Muroga, K. (1985). First records of *Pseudomonas anguilliseptica* infection in cultured ayu, *Plecoglossus altivelis*. Fish Pathol. 20: 481–484

Nakai, T., Muroga, K. (1982). *Pseudomonas anguilliseptica* isolated from European eels (*Anguilla anguilla*) in Scotland. Fish Pathol. 17: 147–150

Nakajima, K., Muroga, K., Hancock, R. E. W. (1983). Comparison of fatty acid, protein and serological properties distinguishing outer membranes of *Pseudomonas anguilliseptica* strains from those of fish pathogens and other pseudomonads. Int. J. Syst. Bacteriol. 33: 1–8

Stewart, D. J., Woldemariam, K., Dear, G., Mochaba, F. M. (1983). An outbreak of 'Sekiten-byo' among cultured European eels, *Anguilla anguilla* L., in Scotland. J. Fish Dis. 6: 75–76

Uno, M. (1976). Experimental pathogenicity of *Aeromonas*

salmonicida, *Vibrio anguillarum* and *Pseudomonas anguilliseptica* for iwana and other trout. Fish Pathol. 11: 5–9

Wakabayashi, H., Egusa, S. (1972). Characteristics of a *Pseudomonas* sp. from an epizootic of pond-cultured eels (*Anguilla japonica*). Bull. Jap. Soc. scient. Fish. 38: 577–587

Wiklund, T., Bylund, G. (1990). *Pseudomonas anguilliseptica* as a pathogen of salmonid fish in Finland. Dis. aquat. Org. 8: 13–19

Wiklund, T., Dalsgaard, I. (1987). Disease outbreaks caused by *Pseudomonas anguilliseptica* in Finnish fish farms. In: Stenmark, A., Malmberg, G. (eds.) Parasites and diseases in natural waters and aquaculture in Nordic countries. Swedish Museum of Natural History, Stockholm, p. 131 (abstract)

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