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NOTE

First isolation of *Cytophaga psychrophila* from a systemic disease in eel and cyprinids

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ABSTRACT: Four *Cytophaga psychrophila*-like strains were isolated from one eel and 3 species of cyprinids with skin lesions and signs of acute septicaemia in Nordrhein-Westfalen (Germany). The strains proved identical in their phenotypic characteristics to each other and to 3 typical strains of *C. psychrophila*, one of which was the type strain. The identification of the new isolates as *C. psychrophila* was confirmed by rapid slide agglutination tests using a specific rabbit anti-*C. psychrophila* serum. This is the first report of *C. psychrophila* being isolated from non-salmonid fishes. This pathogen, which has provoked heavy losses in rainbow trout hatcheries in France and Germany since 1984–1985, may also have the potential to become a problem for non-salmonids. As with most other bacteria pathogenic for fish, *C. psychrophila* shows a lack of strict host specificity.

Cytophaga psychrophila (= *Flexibacter psychrophilus*) was originally isolated from diseased coho salmon *Oncorhynchus kisutch* in Washington State, USA, in 1948 (Borg 1960). This bacterium is the causative agent of a systemic infection known as 'cold-water disease', 'low-temperature disease' or 'peduncle disease'.

Until 1988, the condition had only been reported in the northern states of the USA and in Canada. However, the same bacterium has been recognized since 1988 in western and central Europe as the causative agent of a serious systemic disease in farmed rainbow trout *Oncorhynchus mykiss* (Bernardet et al. 1988, Lehmann et al. 1988).

Until recently, the condition was believed to be restricted to salmonids (*Oncorhynchus* spp., *Salvelinus* spp., and *Salmo* spp.). However, in November 1989, examinations of a wild diseased eel *Anguilla anguilla* from the river Weser in the state of Nordrhein-Westfalen, Germany, revealed the existence of a *Cytophaga psychrophila*-like infection (Lehmann et al. 1990). The eel had a body length of 50 cm, was very lethargic, and showed the following gross lesions: haemorrhages in the unpaired fins, dorsal muscular system, peritoneum, and swim-bladder wall; inflammation of the intestine; and a blister in the region of the anus. The river Weser is highly polluted with NaCl and KCl from salt factories in the east of Germany (average ion concentrations in 1989 at the site where the eel was collected were Na = 860.9 mg l^{-1}, K = 77.2 mg l^{-1}, and Cl = 1661.4 mg l^{-1}). Bacterial infections with motile *Aeromonas* and with *Vibrio anguillarum* are common among eels in this part of the river. The water temperature in November 1989 was about 8 to 10 °C but the rate of mortality among eels during this period was unknown. Virological and parasitic examinations were negative and no bacteria could be isolated on Tryptone Soya agar at 20 °C. However, on Anacker and Ordal agar (AOA) (Anacker & Ordal 1955), a *C. psychrophila*-like organism (i.e. a gliding Gram-negative rod producing bright yellow, non-adherent colonies on AOA) was isolated in pure culture from liver, kidney, and spleen. In imprints of spleen tissue, the bacteria appeared as rather long rods, and occurred especially in granulocytes and leucocytes (Fig. 1).

Other *Cytophaga psychrophila*-like strains were subsequently isolated by the same procedure: in February 1990 from diseased carp *Cyprinus carpio*, in March 1990 from diseased tench *Tinca tinca*, and in April 1990 from diseased crucian carp *Carassius carassius*. All of the fishes were caught in different ponds and artificial lakes in Nordrhein-Westfalen where mixed species culture is common. The water temperature was 8 to 12 °C at the time of collection for all 3 species of fish. There was no indication of water pollution at any of the sites examined. Carp and tench aged 2 to 3 yr had been introduced into the ponds a few weeks earlier, and at the time of collection the mortality among these fish had reached 40 to 50 %. Five fish of each species, exhibiting gross lesions similar to those previously described for the eel, were sampled. The *C. psychrophila*-like organism was isolated in pure culture from the spleen and/or

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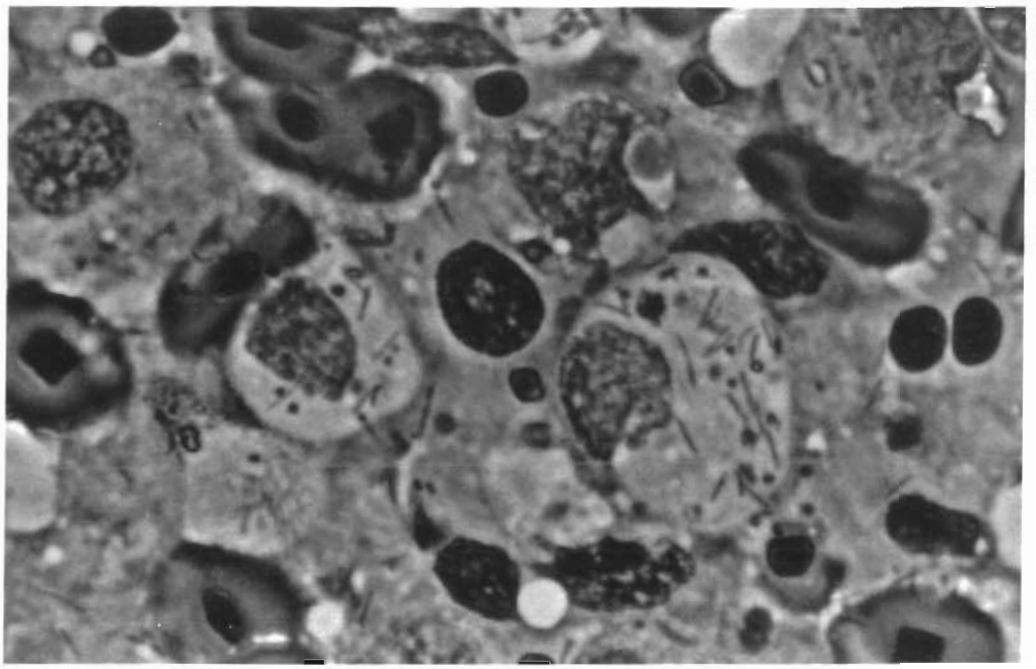

Fig. 1. *Anguilla anguilla* infected with *Cytophaga psychrophila*. Imprint of spleen tissue. Note the numerous bacteria inside the leucocytes. Pappenheim, phase-contrast microscopy, 1000 ×

the liver of all fish. This bacterium was also isolated from crucian carp suffering a mixed septicemic infection with *Aeromonas hydrophila*.

Four strains we isolated (one from the eel, one from a carp, and 2 from tench) were compared to the type strain of *Cytophaga psychrophila* (NCIMB 1947^T, National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland) and to 2 *C. psychrophila* strains isolated from salmonids in France (ATCC 49510 and 49511, American Type Culture Collection, Rockville, USA). The morphological and biochemical tests used have been described previously (Bernardet & Grimont 1989, Bernardet & Kerouault 1989, Bernardet et al. 1990). The results of these tests indicated that all of the new isolates conformed to the description of the bacterial species *C. psychrophila* (Bernardet & Grimont 1989) (Table 1). The following characteristics are particularly significant: colonies on AOA were smooth, glossy, bright yellow, and did not adhere to the agar; as frequently observed for *C. psychrophila*, the 4 isolates produced both circular colonies with regular edges and other colonies exhibiting narrow and uneven spreading margins on the same agar plate (Fig. 2); cells were Gram-negative rods 1 to 5 µm long and 0.3 to 0.5 µm wide in 48 h liquid cultures; in hanging drop preparations, the gliding movement of the bacterial cells in contact with the cover slip (typical of most bacterial species belonging to the order Cytophagales) was usually slow and difficult to observe; gliding was more readily observed directly on agar, at the margin of the spreading zone, by phase contrast microscopy (Henrichsen 1972); the pigments were nondiffusible and belonged to the flexirubin type as demonstrated by a positive result in the KOH test (Reichenbach et al. 1980); colonies did not absorb Congo red and thus did not produce an extracellular galactosamine glycan (McCurdy 1969); catalase and cytochrome oxidase were produced and most clearly shown with young colonies (2 to 4 d); all of the strains tested grew well at 5 °C but did not grow above 26 °C; there was no growth on Tryptone Soya (TS) agar or in TS broth. The enzyme production patterns as tested in API ZYM galleries (API System, La Balme-les-Grottes, France) were identical to the pattern previously published in the description of the species *C. psychrophila* (Bernardet & Grimont 1989). The negative result found for all of the enzymes involved in carbohydrate metabolism (Nos. 13 to 20 in the galleries) was shared in common with *Flexibacter columnaris* and *F. maritimus*. This feature clearly distinguishes these 3 fish-pathogens from the saprophytic or opportunistic Cytophagales commonly isolated from the external lesions of fish. The latter organisms are usually related to *Cytophaga aquatilis* and *C. johnsonae* (Bernardet unpubl.).

A specific antiserum was produced by immunizing a rabbit with formalin-killed cells of the *Cytophaga psychrophila* type strain (NCIMB 1947^T) injected intradermally (A. Paraf pers. comm.). The agglutinating titer of the antiserum was 1/128. Bacterial cells of all the strains studied were cultivated on AOA plates, suspended in saline, and heated for 10 to 15 min at 50 °C to prevent autoagglutination (Holt 1987). Rapid slide agglutination tests showed that the 4 strains isolated from eel and cyprinids in Germany, as well as the 2

Table 1. Characteristics of *Cytophaga psychrophila* type strain (NCIMB 1947[T]), 2 French strains (ATCC 49510 and 49511), and 4 German test strains (+: all strains give a positive reaction; (+): weak positive reaction; −: negative reaction)

Tests	Results
Morphology of colonies on AOA[a]	Bright yellow, convex, circular and/or weakly spreading
Adherence to the agar	−
Gliding motility in AOB[a]	(+)
Flexirubin-type pigment[b]	+
Congo red absorption[c]	−
Anaerobic growth[d]	−
Presence of cytochrome oxidase[e]	+
Presence of catalase	(+)
Nitrate reduction[f]	−
H_2S production[g]	−
Presence of β-galactosidase[h]	−
Hydrolysis of cellulose[i]	−
carboxymethylcellulose[j]	−
chitin[k]	−
starch[l]	−
gelatin[m]	+
casein[n]	+
tyrosine[o]	+
Production of brown diffusible pigment on tyrosine agar	−
Hydrolysis of tributyrin[p]	+
lecithin[q]	+
Tween 20[r]	+
Tween 80[r]	+
DNA[s]	+
Growth in trypticase-soy agar and broth	−

[a] AOA and AOB = Anacker and Ordal Agar and Broth (Anacker & Ordal 1955); [b] Detected by flooding the colonies with 20 % (W/V) KOH (Reichenbach et al. 1980); [c] Detection of an extracellular galactosamine glycan (McCurdy 1969); [d] Growth in deep AOA tubes; [e] Commercially prepared test paper (Diagnostics Pasteur, Marnes-la-Coquette, France); [f] 0.1 % potassium nitrate AOB tubes (Bullock 1972); [g] Lead acetate paper (Pacha 1968); [h] *o*-nitrophenyl-β-D-galactopyranoside commercially prepared test paper (Diagnostics Pasteur); [i] Sterile filter paper on Dubos agar (Reichenbach & Dworkin 1981); [j] 3 % carboxymethylcellulose AOB (modified from Lewin & Lounsbery 1969); [k] 20 % Chitin AOA (Reichenbach & Dworkin 1981); [l] 0.2 % soluble starch AOA (Bullock 1972); [m] Film method (Le Minor & Piechaud 1963); [n] 5 % skim milk AOA (modified from Pacha 1968); [o] 0.5 % L-tyrosine AOA (Bullock 1972); [p] 1 % glycerol tributyrate and 0.1 % polyvinylic alcohol agar (Mourey & Kilbertus 1976); [q] Precipitate on 5 % sterile egg yolk AOA (modified from Cowan & Steel 1974); [r] 1 % Tween 20 or 80 peptone agar (modified form Sierra 1957); [s] Commercially prepared DNA agar (Diagnostics Pasteurs)

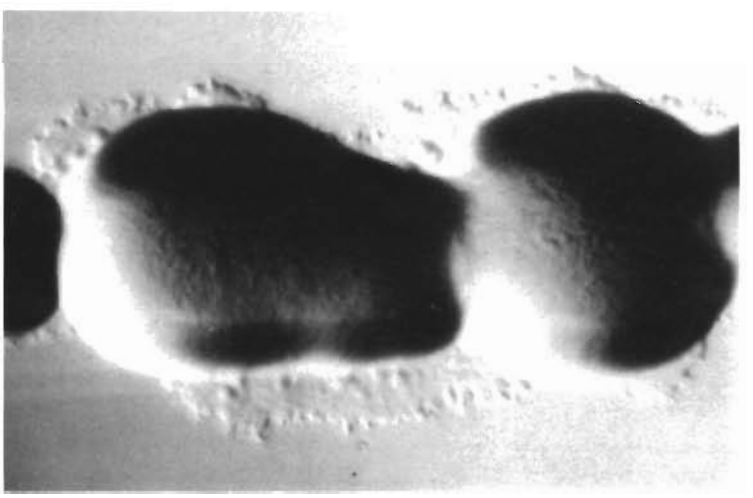

Fig. 2. *Cytophaga psychrophila*. Colonies of a strain isolated from diseased tench in Germany. Note the uneven spreading margins originating from the edges of the circular colonies. Anacker and Ordal agar, 20 °C, 5 d. Diameter of colonies is 1 mm

French strains isolated from salmonids, reacted strongly with the specific anti-NCIMB 1947^T serum, whereas there was no agglutination either with the serum from a non-immunized rabbit or with saline only. The antiserum did not agglutinate the cells of *Flexibacter columnaris* NCIMB 2248^T, *F. maritimus* NCIMB 2154^T, or 4 strains related to *C. aquatilis* and *C. johnsonae* that we had isolated from external lesions of fish.

The phenotypic properties of the *Cytophaga psychrophila*-like strains isolated from the diseased eel and the cyprinids in Germany are therefore identical to those of the type strain of the species and to the *C. psychrophila* strains previously isolated from salmonids. The serological similarity to the type strain of the species is one more argument for their identification as *C. psychrophila*. These studies are the first to show that *C. psychrophila* can infect non-salmonid fishes. Experimental studies are needed to confirm the pathogenicity of the 4 isolates because several other factors may have contributed to the observed disease. These include the pollution of the river Weser, the recent introduction of (and the consequent stress to) the carp and tench in the ponds, and the presence of other bacterial species such as *Aeromonas* and *Vibrio* spp. If these strains are found to be pathogenic to non-salmonid fishes (e.g. Anguillidae and Cyprinidae), it may indicate that *C. psychrophila* will become more important as a pathogen in Europe. The severity of the problem for salmonids has already been observed in several rainbow trout hatcheries in Nordrhein-Westfalen and in western and southwestern France. Furthermore, the spread of *C. psychrophila* by non-salmonid fishes with apparent or inapparent infections could be an important factor in the epizootiology of cold-water disease among salmonid populations in Europe. Finally, the present findings with *Cytophaga psychrophila* provide another example of the lack of host specificity among bacteria pathogenic for fish.

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