Short communication

Isolation of *Flavobacterium psychrophilum* from sea lamprey, *Petromyzon marinus* L., with skin lesions in Lake Ontario

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Diseases caused by *Flavobacterium psychrophilum*, a Gram-negative, long rod (0.2–0.75 × 1.5–7.5 μm) have recently become one of the most crucial problems affecting salmonid culture worldwide (Wood & Yasutake 1956; Pacha 1968; Lorenzen & Olesen 1997). In North America, the disease is known as cold water disease (CWD) because of its occurrence usually at low water temperature among different species of salmonids. The disease is characterized by skin erosions and ulcerations, darkening, fin and gill rot. In severe cases, the caudal fin is completely sloughed with the bare spine in the caudal peduncle area being completely exposed. In very young fish, the disease can also be associated with nervous manifestations such as erratic swimming behaviour and spiral movements (Holt, Amand, Rohovec & Fryer 1989; Kent, Groff, Morrison, Yasutake & Holt 1989).

*Flavobacterium psychrophilum* has been reported in all salmonid species (Cipriano & Holt 2005). The bacterium has also been reported from other non-salmonid species including ayu, *Plecoglossus altivelis* (Temminck & Schlegel) (Lee & Heo 1998); common carp, *Cyprinus carpio* L.; crucian carp, *Carassius carassius* (L.); eel, *Anguilla anguilla* (L.) (Lehmann, Mock, Stuerenberg & Bernardet 1991); perch, *Perea fluviatilis* L.; and roach, *Rutilus rutilus* (L.) (Madetoja, Dalsgaard & Wiklund 2002). However, the bacterium has never been reported from the sea lamprey and the potential role played by the sea lamprey in the dissemination of the bacterium to cohabitant salmonids has not been addressed.

In the Great Lakes basin, sea lamprey, *Petromyzon marinus* L., has been called the most destructive of invasive species (Lupi & Hoehn 1998). Adult parasitic sea lamprey have been incriminated as a major factor contributing to the collapse of the lake trout, *Salvelinus namaycush* (Walbaum), and the lake whitefish, *Coregonus clupeaformis* (Mitchill), fisheries in the Great Lakes during the early 1940s and 1950s (Smith & Tibbles 1980). To reduce the number of sea lamprey and limit its spread, the Great Lakes Fishery Commission began a large-scale programme based on male sterilization and release to compete with fertile males during spawning. Males are collected from different sites at Lake Ontario, transported into a sterilizing facility in Hammond Bay, Michigan, and then released into selected river systems basin-wide. These relocation procedures may transfer various pathogens among different fish populations, which
raise major concerns regarding the possibility of
introducing detrimental infections into non-
exposed fish populations in the Great Lakes.

In the current study, we report isolation of
_F. psychrophilum_ from sea lamprey associated with
various skin lesions. This report is considered the
first report of _F. psychrophilum_ from sea lamprey.
Data shown in the current study might shed light
on the potential role of the sea lamprey in spreading
_F. psychrophilum_ infections to their cohabitant
salmonids in the Great Lakes basin.

In midsummer 2004, 118 adult sea lamprey were
transported alive from the Humber River and
Duffins Creek, Lake Ontario, to the Aquatic
Animal Health Laboratory at Michigan State
University for health inspection to determine their
suitability for transfer to the lamprey sterilizing
facility at Hammond Bay. Lampreys were examined
externally and internally for the presence of lesions
or parasites. Bacteriological swabs from lesions were
cultured onto tryptic soy agar (TSA; Remel, Lenexa,
KS, USA) and Hsu-Shotts agar (Bullock, Hsu &
Shotts 1986). Inoculated agar plates were incubated
at 15 °C and checked daily for up to 5 days. Two
isolates were observed on Hsu-Shotts agar but not
on TSA and presumptively identified as _F. psychro-
philum_ according to morphological and biochemical
criteria (Pacha 1968; Lorenzen, Dalsgaard &
Bernardet 1997). Further biochemical testing was
performed using API 20NE tests (BioMerieux Inc.,
Durham, NC, USA) which were incubated at
15 °C and interpreted at 48–72 h according to the
manufacturer’s instructions.

Chromosomal DNA was extracted from 100 μL
bacterial suspension (single colony of each of the
isolated bacteria suspended in 100 μL sterile saline)
using a DNeasy tissue extraction kit (Siege Inc.,
Valencia, CA, USA) according to the manufactur-
mer’s instructions. The extracted DNA was amplified
using an oligonucleotide primer set specific for
_F. psychrophilum_ (Urdaci, Chakroun, Faure &
Bernardet 1998; del Cerro, Marquez & Guijaro
2002). The sequence of the two primers was:
primer-1 (5’-CTT AGT TGG CAT CAA CAC-3’)
and primer-2 (5’-ACA CTG GCA GTC TTG
CTA-3’). The controls consisted of a polymerase
chain reaction (PCR) mixture without DNA template
(negative control) and with DNA extracted
from known _F. psychrophilum_ (positive control).
Thermal cycling was performed with a Robocycler
Gradient 96 (Stratagene, La Jolla, CA, USA) with
the amplification conditions according to del Cerro
et al. (2002). The PCR products were electrophore-
sed in 2% agarose gel (Invitrogen Corporation,
Carlsbad, CA, USA), stained with ethidium bromide,
viewed with ultra violet light and photo-
graphed using the Kodak EDAS System (Eastman
Kodak Company, Rochester, NY, USA). Samples
were considered positive when a 971 bp PCR
product specific for _F. psychrophilum_ was detected.

Clinical examination of sea lamprey from Duf-
fins Creek revealed skin lesions in the form of
shallow skin ulcers and erosions in the dorsal and
caudal fins (Figs 1 & 2). Some lamprey showed
severe skin darkening with a white slimy film
covering the inner wall of the nostrils and the eyes.
_Flavobacterium psychrophilum_ was isolated from two
lamprey showing skin lesions of 118 examined
(1.7%). One of the _F. psychrophilum_ isolates was
isolated from the white film covering the nostrils and
the other one was isolated from a dorsal fin
erosion. However, it is crucial to note that _F. psy-
chrophilum_ was isolated from lampreys collected

![Figure 1](image1.png)

_Figure 1_ Sea lamprey showed severe erosion on the ventral aspect of the caudal fin caused by _Flavobacterium psychrophilum_ infection. Note the colouration of the margin of the erosion indicating the chronic nature of the lesion.

![Figure 2](image2.png)

_Figure 2_ Sea lamprey showed severe erosion on the dorsal fin caused by _Flavobacterium psychrophilum_ infection. Note the colouration of the haemorrhagic margin of the erosion indicating the acute nature of the lesion.
from Duffins Creek. Lampreys collected from the Humber River did not show any clinical abnormalities and no bacteria were isolated from them. This has epidemiological significance because the per cent of *F. psychrophilum*-positive lampreys is then increased to 3.45% from Duffins Creek (two of 58) whilst Humber River lampreys were negative (none of 60).

The two isolates were identified as *F. psychrophilum* using both conventional and molecular techniques. On Hsu-Shotts medium, the two isolates produced bright yellow colonies 2–3 mm in diameter with thin spreading margins. Phenotypically, the two isolates were Gram-negative, long bacilli, motile by gliding, very weakly catalase positive, weakly cytochrome oxidase positive, gelatinase test positive, non-agarolytic and produced flexirubin upon addition of 20% KOH (colonies turned brown orange). No growth was observed on TSA plates at 15 or 20 °C or in the presence of 2% NaCl. The two isolates showed optimal growth at 15 °C and there was no growth at 30 or 37 °C. Performing the O/F test using glucose as a sole source of carbon revealed that the two isolates were negative. The two isolates were not able to utilize sucrose, starch or glucose in their basal media. Results of BioMerieux API 20NE rapid test strip inoculations were consistent with the biochemical reactions of *F. psychrophilum* (Cipriano & Holt 2005). Molecular confirmation of the two isolates was performed by using a *F. psychrophilum* species-specific primer set targeting the 16S rRNA genes. The amplification of PCR product of the expected size (971 bp) confirmed the entity of the isolated bacteria as *F. psychrophilum* isolates (Fig. 3).

In conclusion, the current study reports a new non-salmonid host for *F. psychrophilum* infection for the first time in the Great Lakes and worldwide. The affected lampreys showed skin lesions similar to those reported in salmonids during CWD infection. Furthermore, the long-term natural cohabitation between clinically infected parasitic lampreys and salmonids residing in the same water suggests a potential for the spread of *F. psychrophilum* through the Great Lakes basin fisheries. Despite the broad host range of *F. psychrophilum* in North America, it is difficult to assess the impact of lamprey infection on transmission of the infection and its subsequent effects on salmonid fish populations in the Great Lakes. Further studies are required to study the molecular similarities of the isolated *F. psychrophilum* strains with those from salmonids and to assess their pathogenicity to salmonids. Without conclusive evidence of the impacts of this infection, the risk–benefit margins of lamprey transfer or relocation programmes are uncertain.

**References**


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