The seven loci of the *Flavobacterium psychrophilum* MLST scheme and the PCR and sequencing protocols.

Locus	Forward primer (5'-3')	Reverse primer (5'-3')	Length[a]
trpB	CAGGAAACAGCTATGACCAAGATTATGTAGGCCGCCC	TGTAAAACGACGGCCAGTTGATAGATTGATGACTACAATATC	789 bp
gyrB	CAGGAAACAGCTATGACCGTTGTAATGACTAAAATTGGTG	TGTAAAACGACGGCCAGTCAATATCGGCATCACACAT	1077 bp
dnaK	CAGGAAACAGCTATGACCAAGGTGGAGAAATTAAAGTAGG	TGTAAAACGACGGCCAGTCCACCCATAGTTTCGATACC	873 bp
fumC	CAGGAAACAGCTATGACCCCAGCAAACAAATACTGGGG	TGTAAAACGACGGCCAGTGGTTTACTTTTCCTGGCATGAT	750 bp
murG	CAGGAAACAGCTATGACCTGGCGGTACAGGAGGACATAT	TGTAAAACGACGGCCAGTGCATTCTTGGTTTGATGGTCTTC	681 bp
tuf	CAGGAAACAGCTATGACCGAAGAAAAAGAAAGAGGTATTAC	TGTAAAACGACGGCCAGTCACCTTCACGGATAGCGAA	795 bp
atpA	CAGGAAACAGCTATGACCCTTGAAGAAGATAATGTGGG	TGTAAAACGACGGCCAGTTGTTCCAGCTACTTTTTTCAT	834 bp

[a] Length of the target sequence.

forward sequencing primer: 5′-CAGGAAACAGCTATGACC-3′
reverse sequencing primer: 5′-TGTAAAACGACGGCCAGT-3′

PCR and sequencing protocols

From: Erina Fujiwara-Nagata et al. Population Structure of the Fish Pathogen *Flavobacterium psychrophilum* at Whole-Country and Model River Levels in Japan (submitted).

F. psychrophilum isolates were grown in MCYT broth for 2 days at 15°C and 140 rpm and the genomic DNA was extracted from the pellet using the Wizard Genomic DNA purification kit (Promega). PCR amplification was performed in a 20-ìL reaction volume using GoTaq polymerase (Promega) and the following touchdown protocol: 94°C for 5 min; 24 cycles at 94°C for 0.5 min, 55°C for 0.5 min (-0.4°C/ cycle), and 72°C for 1 min (+2 sec/ cycle); 12 cycles at 94°C for 0.5 min, 45°C for 0.5 min, and 72°C for 2 min (+3 sec/ cycle); and a final extension step at 72°C for 10 min. Five microliters of the PCR products was resolved on a 1% agarose/TBE gel to check amplification. For

sequencing, one microliter of the PCR products was purified by using exonuclease I (Biolabs)-alkaline phosphatase (USB) for 1 h at 37°C, followed by enzyme inactivation for 5 min at 94°C. One-tenth of the purified PCR products was sequenced on both strands, using the sequencing primers, the BigDye Terminator version 3.1 sequencing kit (Applied Biosystems), and an Applied Biosystems 3730 automated sequencer.