

Flavobacteriosis, the main disease problem for fish farming in Sweden

Eva Jansson, Anders Alfjorden, Eva Säker, Olga Stephansson, Sofia Lindström & Anna Aspán

National Veterinary Institute (SVA), Section of Fish, SE-75189, Uppsala, Sweden

Correspondence: eva.jansson@sva.se

Abstract

Infection with *Flavobacterium psychrophilum* is the most common reason for antibiotic therapy in Swedish aquaculture today. High mortalities are reported especially in rainbow trout, *Oncorhynchus mykiss*, fry and fingerlings and this species seems to be more affected compared with Arctic char, *Salvelinus alpinus*, brown trout, *Salmo trutta*, and Atlantic salmon, *Salmo salar* (Fig. 1). Severe lesions in form of ulceration of the peduncle and tail fins, sometimes deep into the musculature and into the skeleton, are common signs of disease. Fish become anemic and the infection often results in an acute septic anemia. Chronic infections in adults, exhibiting haemorrhagic areas in the skin, muscle lesions and blood-filled abscesses, have occurred. Infections with *F. columnare* are regularly detected during summer months at elevated water temperatures, in fish with gill and skin lesions. Brown trout are more frequently affected compared with Arctic char, rainbow trout and salmon (Fig. 2). Gram-staining of smears from tissues was in the middle of the 1980's the main technique for diagnosis of flavobacteriosis and the characteristic yellow colonies of *F. psychrophilum* were also occasionally isolated on Cytophaga agar [1]. *F. columnare* was for the first time isolated in 1996. Sporadic isolations of *F. psychrophilum* have also been made in Sweden from eel, *Anguilla anguilla*, carp, *Cyprinus carpio*, perch, *Perca fluviatilis*, ornamental fish, grayling, *Thymallus thymallus*, tench, *Tinca tinca*. *F. columnare* have been detected also in carp and perch.

Isolates of *F. psychrophilum* and *F. columnare* from the strain collection of the National Veterinary Institute (SVA), together with bacterial samples collected during natural outbreaks at fish farms, were used in an evaluation of the efficacy of Cytophaga agar [2], kidney disease medium charcoal (KDM-C) agar [3] and trypton yeast extract salt (TYES) agar [4] for isolation of these species. This study demonstrated TYES agar to be superior for isolation of *F. columnare* (Fig. 3a,b), but no difference in recovery of *F. psychrophilum* was demonstrated, (Fig. 4a,b). The bright yellow colonies of *F. psychrophilum* were however easily visible on the black KDM-C agar, especially useful for identification of the bacterium from skin and fin samples where mixed cultures are common.

Florfenicol is the main substance used for treatment of flavobacteriosis in Sweden. Sensitivity to antibiotics is evaluated by determination of the minimal inhibitory concentration (MIC)-value. A standardized inoculum of the bacterium is added to each well of microtitre-plates containing dilutions of the antimicrobial. The endpoint of growth is decided, giving the MIC value (VetMic, Sweden). A panel of different antimicrobials is included on each plate, this allow the possibility to follow the sensitivity pattern of several compounds. According to the classification of antibiotic sensitivity, established for warm-blooded animals, the cut off value for Florfenicol is set to a MIC-value of 16 mg/L. All isolates of *F. psychrophilum* ($n=141$) and *F. columnare* ($n=69$) tested since 2005 were according to this, classified as sensitive. The distribution of MIC-values for Nalidixin acid (oxolinic acid) show however several isolates

with MIC-value above the cut-off value indicating the presence of isolates with a reduced susceptibility to oxolinic acid.

Thirty-eight Swedish isolates of *F. psychrophilum* collected from diseased fish between 1988 and 2011 were characterized by Multi Loci Sequence Typing (MLST) [5], i.e. by a comparison of seven genes (*atpA*, *dnaK*, *fumC*, *gyrB*, *murG*, *trpB* and *tuf*). An eBurst analysis demonstrated that the majority of isolates were closely related (Fig. 5). This comparison was based on somewhat shorter DNA sequences compared with the protocol from the *F. psychrophilum* MLST Database at Pasteur. The study indicated a high homogeneity among Swedish isolates of *F. psychrophilum* which supports that no new strains have been introduced over time.

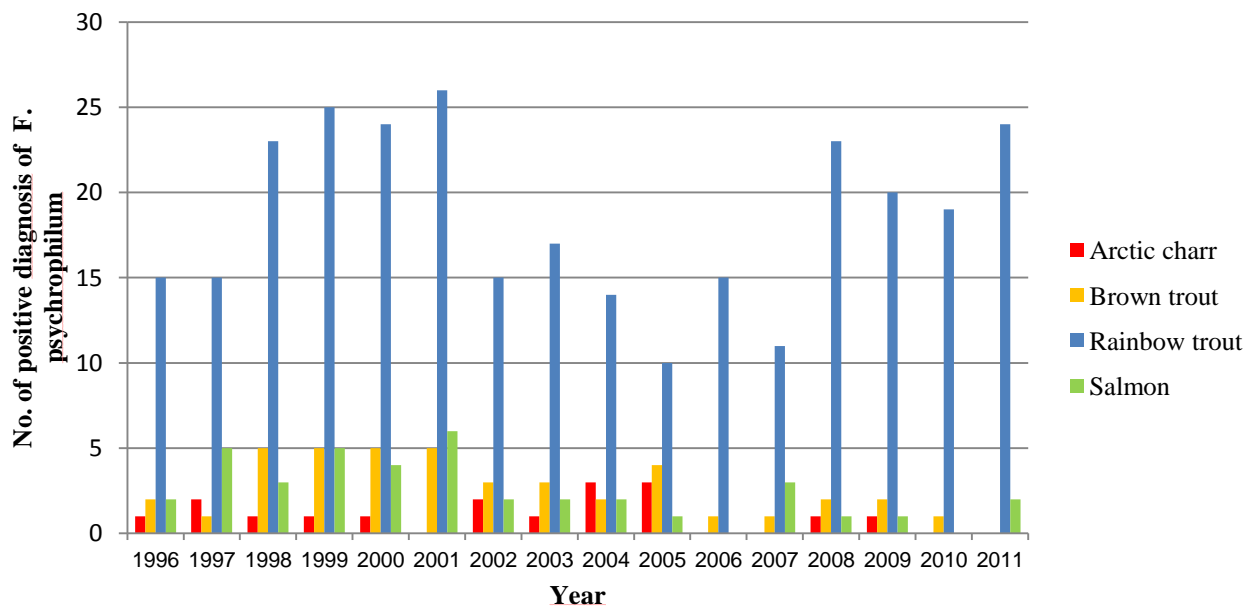


Figure 1. *F. psychrophilum* isolated in Swedish fish farms in connection with disease 1996-2011.

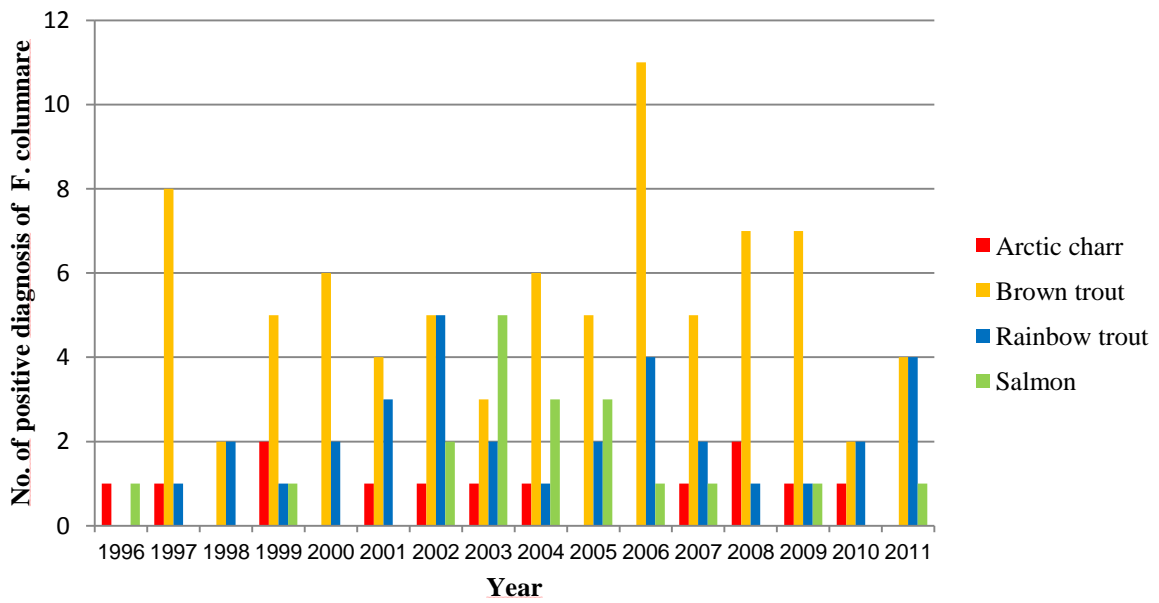


Figure 2. *F. columnare* isolated in Swedish fish farms in connection with disease 1996–2011.

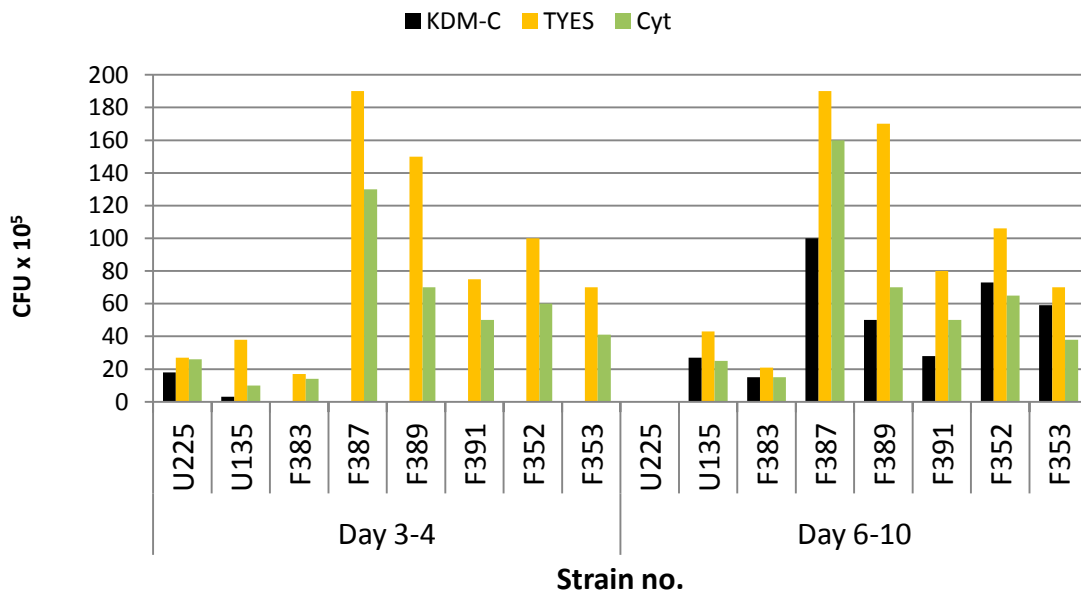


Figure 3a. Serial dilutions of laboratory isolates of *F. columnare* inoculated on KDM-C agar, TYES agar and Cytophaga (Cyt) agar at 20 °C.

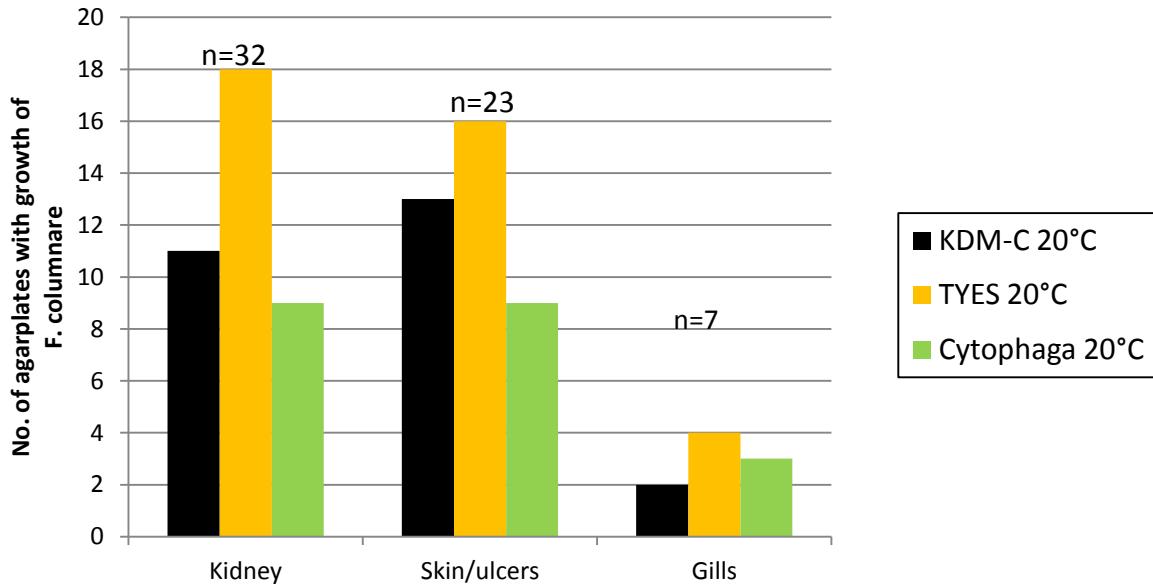


Figure 3b. Evaluation of agar for primary isolation of *F. columnare* on KDM-C agar and TYES agar at 20 °C. Samples collected during field conditions.

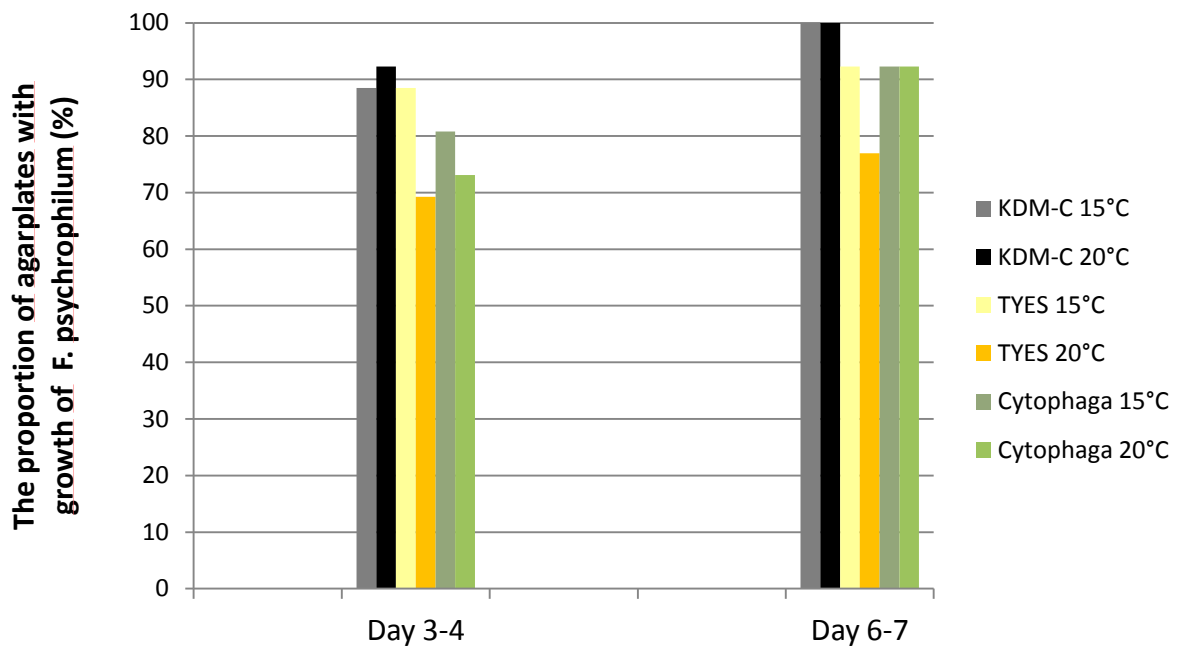


Figure 4a. The proportion of agar plates with growth of laboratory strains of *F. psychrophilum* (n=26) on KDM-C agar, TYES agar and Cytophaga agar at 15 or 20 °C.

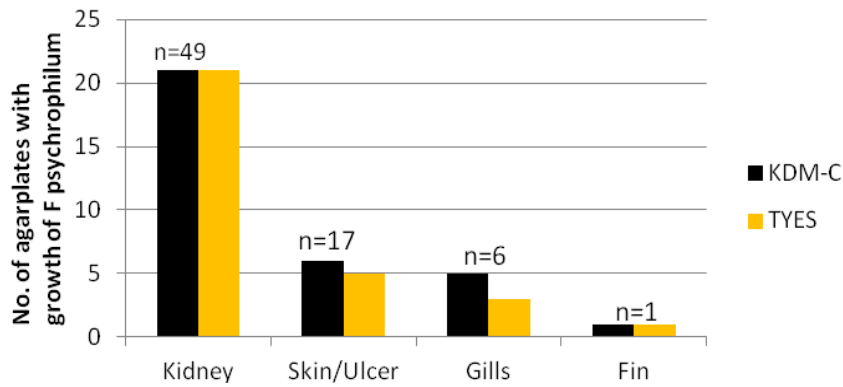


Figure 4b. Evaluation of agar for primary isolation of *F. psychrophilum* on KDM-C agar and TYES agar at 20 °C. Samples collected during field conditions.

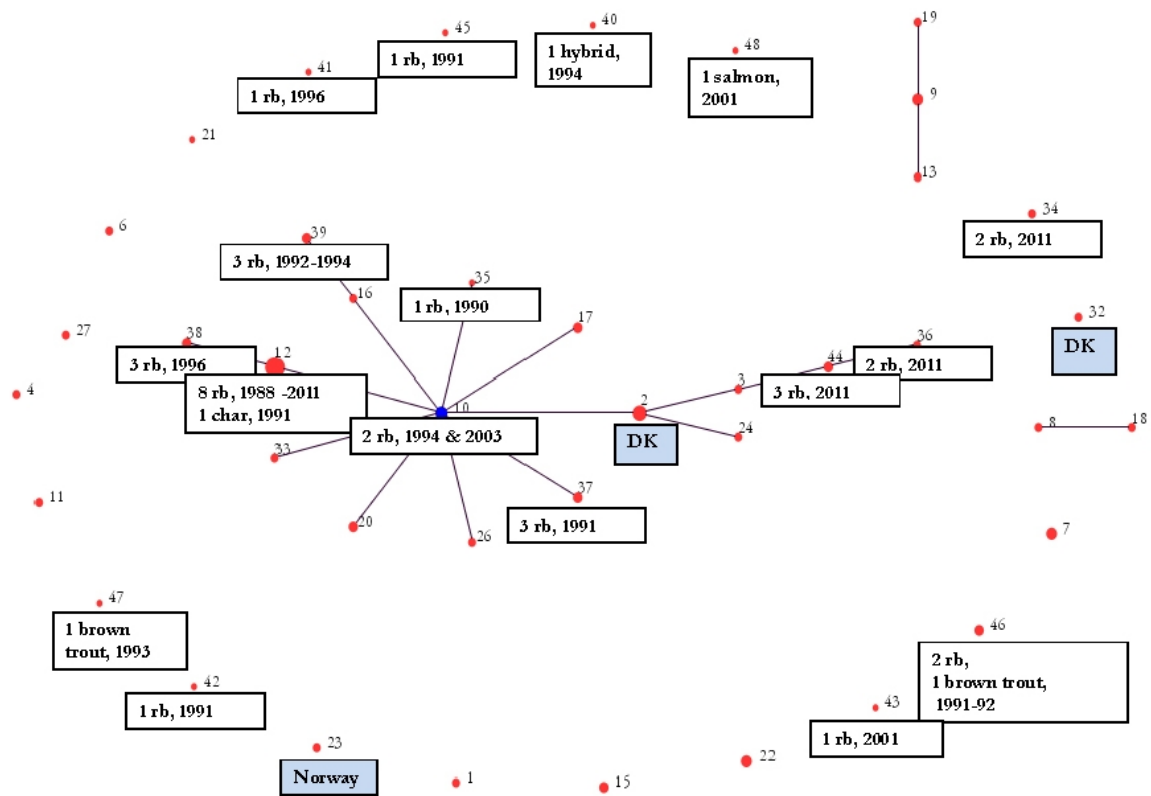


Figure 5. Multi Loci Sequence Typing (MLST), comparing 7 reference loci. Each square represent 1 up to 8 Swedish isolates from rainbow trout (rb), brown trout, char or a hybrid (“splake”: *Salvelinus fontinalis* × *Salvelinus namaycush*). Remaining dots represents strains from the on line database.

References

- [1] Anacker R., Ordal E., 1955. Study of a bacteriophag infecting the myxobacterium *Chondrococcus columnaris*. *Journal of Bacteriology*, 78:25–32
- [2] Bernardet J.-F., Kerouault B., 1989. Phenotypic and genomic studies of *Cytophaga psychrophila* isolated from diseased rainbow trout (*Oncorhynchus mykiss*) in France. *Applied and Environmental Microbiology*, 55:1796–1800
- [3] Daly J., Stevensson R., 1985. Charcoal agar, a new growth medium for the fish disease bacterium *Renibacterium salmoninarum*. *Applied and Environmental Microbiology*, 50:868–871
- [4] Holt R., Rohovec J., Fryer J., 1993. Bacterial coldwater disease. In: Inglis V., Roberts R.J., Bromage N.R. (Eds.) *Bacterial diseases of fish*. Blackwell Scientific Publications, Oxford, UK. 3–23
- [5] Nicolas P., Mondot S., Achaz G., Bouchenot C., Bernardet J., Duchaud E., 2008. Population structure of the fish-pathogenic bacterium *Flavobacterium psychrophilum*. *Applied and Environmental Microbiology*, 74:3702–3709