FLAVOBACTERIUM CAUSED FISH DISEASE IN HUNGARY

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Flavobacterium columnare is a ubiquitous bacterium in the aquatic environment. As the etiological agent of columnaris disease it affecs the skin and gills both of wild and cultured freshwater fishes. This aquatic pathogen causes severe mortalities and economic losses for the fishing industry throughout the world. It favours warm water and cause problems in Hungarian fish ponds during the summer months. Increasing temperature of the freshwaters, by the global warming, elevates the risks of this disease also in natural waters..

The aim of our study was to survey the prevalence of *Flavobacterium columnare* in fishes both from wild and cultured freshwater in Hungary. The samples were collected from the skin, eye, gill and visceral organs of diseased fish and from gills of healthy specimens.

For the selective isolation, the low nutrient and high water content Cytophaga agar supplemented with neomycin and polymixin-B was used. The culturing of the isolates was carried out on the same media without antimicrobial agents. In the preliminary examinations we studied the morphology, motility, staining characteristics and biochemical features of the bacterial colonies. Identification of the isolates was confirmed with specific PCR based on 16S rRNA gene of *F. columnare* (Bader et al., 2003). In the latter assay 25 isolates from 10 different fishes (common carp, gibel carp, tench, razorfish, common bream, silver bream, perch, sander, Volga sander, and Siberian sturgeon) gave positive result. Henceforth the genotypes of the strains were determined by restriction fragment length polymorphism (RFLP) of 16S rRNA gene fragment using HaeIII and RsaI restriction endonucleases (Darwish et al. 2005). Both the original fragment size and RFLP band patterns of our strains differed from the published ones. Twenty of 25 strains presented identical RFLP pattern, while four others varied from them using either of HaeIII or RsaI restriction endonucleases, and the last one differed from all of them using with both enzymes.

The sequence analysis of about 1360 bp long 16S rRNA gene fragment identified 23 strains as a *F. johnsoniae,* closely related species of *F. columnare,* meanwhile the remaining 2 strains proved to be *Chryseobacterium* spp. Antibiograms of the strains was determined by Kirby-Bauer disc diffusion method using with 10 antimicrobial agents. The lack of international guide specific for *Flavobacterium* made the evaluation of the results difficult. The strains were multiresistant. All of them exhibited resistance to ampicillin and polymyxin B,

while the 23 *F. johnsoniae* isolates were resistant also to gentamycin. High level of antimicrobial resistance was revealed to chloramphenicol as well. Only 3 separated *F. johnsoniae* strains demonstrated sensitivity to this antimicrobial agent and did it with a *Chryseobacterium* to oxytetracycline. Prevalences of resistance and sensitivity to furazolidone and cotrimoxazole of the strains were similar, with 11 and 13 resistant strains, respectively. Florfenicol, enrofloxacin and erythromycin were more effective against our isolates and only 4 strains were resistant to them.

Our results indicate that the genus *Flavobacterium* were represented by *F. johnsoniae* in addition to *Chryseobacterium* spp both in wild and cultured freshwater fishes during the studied period. Considering the prevalence of high level multiresistant strains in diseased fishes, further examinations of their epidemiological role and relation to the public health are needed.

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