Preliminary Observations of Pathological Changes to Skin and Gill of Channel Catfish (*Ictalurus punctatus*) and Zebra Danio (*Danio rerio*) Exposed to *Flavobacterium columnare*

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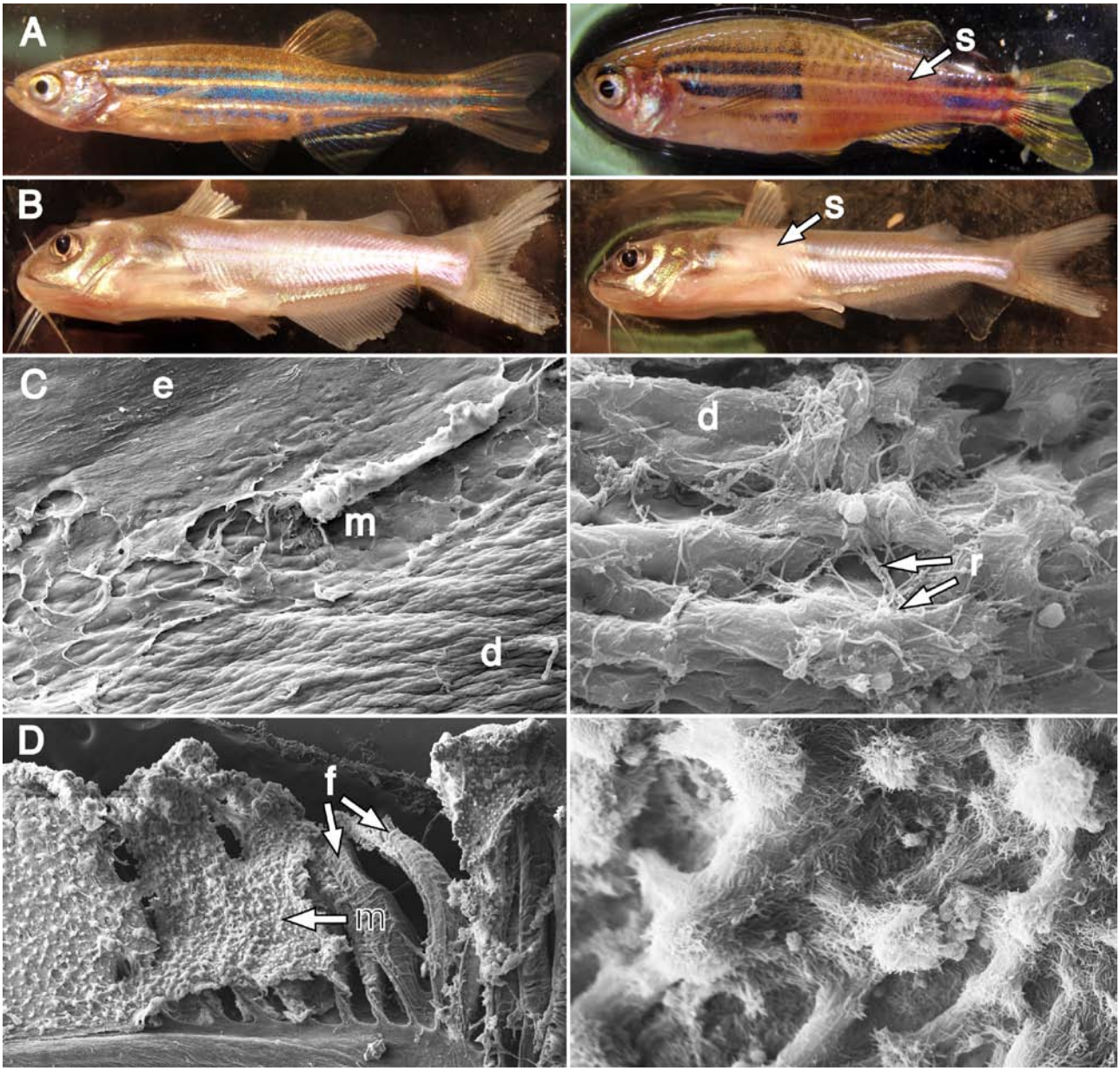
Abstract

Although *Flavobacterium columnare* is the causative agent of "columnaris disease" and comprises a serious pathogen of cultured freshwater fishes (1) few details are available regarding specific pathological changes to fish tissue associated with acute and chronic stages of infection; especially among different fish species exposed to different strains. The first step to study columnaris pathogenesis depends upon the availability of a reliable challenge model; however, this is difficult since bacterial strain, water quality, and fish disposition may synergistically affect bacterium virulence. We have routinely used several highly virulent strains of *F. columnare* in combination with an immersion challenge model to study pathogenesis among specific pathogen free (SPF) fingerlings of channel catfish, *Ictalurus punctatus*. However, availability of SPF channel catfish fingerlings became logistically prohibitive because this catfish species spawns once annually and we required fingerlings because they are small and logistically easier to process for histopathology. To optimize our challenge model, we incorporated zebra danios (*Danio rerio*), also called "zebrafish," because adults are small and fit into a standard-sized tissue cassette, can be obtained year round as "laboratory reared," are inexpensive, and are easily maintained in laboratory settings. Fish were starved 4 days before challenge, exposed to three strains of *F. columnare* (10^6 CFU/ml) for 30 minutes, placed into separate 30 L tanks at 26°C in a 12 hour light/dark cycle (15 *I. punctatus*, 30 *D. rerio* per tank; 3 tanks per strain and another 3 tanks were controls), and observed for behavioral changes and mortality over a 7-day time course. Gill, skin, and viscera of moribund or recently-dead fish were opportunistically sampled for histopathology and scanning electron microscopy (SEM), and separate challenges were used for each fish species. Although strain differences in pathogenesis remain indeterminate, gross signs of infection in individuals of both fishes were comparable and manifested within 12-96 hours. Infected fish generally congregated near the water surface, were less active and relatively inappetent as compared to controls. Grossly, the skin lesion appeared as a pale or whitish lateral band on the trunk (Fig. 1A, B), probably indicative of epidermal sloughing (Fig. 1C). SEM revealed rod shaped bacteria on the presumptive exposed dermis (Fig. 1C). In zebra danio, SEM revealed that some scales were nearly denuded of epidermis. Although scale loss from *D. rerio* was not observed, we suspect it may occur in chronic infections because *F. columnare* has been associated with scale loss in other species (1). The above observations confirm previous reports (2) that the "saddleback" lesion may initially be an erosive condition. Regarding gill, *F. columnare* is thought to infect respiratory epithelium (1), and the probability for adhesion to gill could theoretically be increased by the serial redundancy of filaments and lamellae that create a high surface area for bacterial adhesion. Grossly, gills of challenged and control fish were bright red and appeared normal upon removal. Histologically, both fish exhibited probable hyperplasia of gill epithelium, and, in some areas, these epithelial cells filled interlamellar water channels, resulting in apparent "clubbing" of some filaments. SEM observations of the gill of both *I. punctatus* and *D. rerio*

suggested that *F. columnare* may form a mat-like coating that envelops gill filaments (Fig. 1D). SEM also revealed many nodules comprising large numbers of coalescing rod-shaped bacteria that were associated with this coating (Fig. 1D). Aggregations of *F. columnare* have been previously referred to in the literature as "haystacks" (3), and our data may represent the first SEM observation of haystack-like nodules on the gills. In general, and considering these preliminary observations, we regard gross, SEM, and histopathological comparisons between these fish species challenged with different strains of *F. columnare* as a promising area of further study regarding pathogenesis and ecology of *F. columnare*.



(A) Normal, uninfected zebra danio (*Danio rerio*) (left) aside infected *D. rerio* (right) with "saddleback" lesion (s). (B) Uninfected channel catfish (*Ictalurus punctatus*) (left) aside infected *I. punctatus* (right) with "saddleback" lesion (s). (C) Scanning electron micrograph of a "saddleback" lesion showing presumed epidermis (e), lesion margin (m), and presumed exposed dermis (d) (left, 150×), and filamentous rod shaped bacteria on exposed dermis (right, 4,500×). (D) Scanning electron micrograph of infected *I. punctatus* gill with a mat-like covering (m) on several gill filaments (f) (100×), and nodules ("haystacks") comprising aggregations of filamentous rod shaped bacteria (right, 2,000×).

References

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