Vol. 8: 73–75, 1990 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published March 6

NOTE

Scanning electron microscopy on the skin surface of ayu *Plecoglossus altivelis* infected with *Vibrio anguillarum*

T. Kanno, T. Nakai, K. Muroga*

Faculty of Applied Biological Science, Hiroshima University, Higashi-Hiroshima 724, Japan

ABSTRACT: A scanning electron microscopic study of ayu *Plecoglossus altivelis* infected experimentally with *Vibrio anguillarum* showed large numbers of bacterial cells on the skin surface in advanced stages of the infection. These cells on the skin surface may serve as an important source of the infection among cultured ayu.

Vibriosis, caused by *Vibrio anguillarum*, results in substantial mortality in cultured ayu *Plecoglossus altivelis* in freshwater ponds in Japan (Muroga & Egusa 1988). Recent works on the infection mechanism of vibriosis in ayu indicated that seed ayu carrying *V. anguillarum* could serve as a reservoir of the infection in ponds (Muroga et al. 1984). Bacteria shed from diseased fish were transmitted to healthy ones by the waterborne route (Kusuda et al. 1981, Kanno et al. 1989). Histological investigations revealed that the first *V. anguillarum* colonization site in ayu was the skin (Funahashi et al. 1974, Muroga & De La Cruz 1987), and in another study (Kanno et al. 1989), it was demonstrated, using a patch contact challenge method, that the skin, fins, and anus were portals of entry for the pathogen. In the present study, we used bacteriological culture and scanning electron microscopy (SEM) to describe the fate of *V. anguillarum* in or on the skin of experimentally infected ayu.

Materials and methods. A virulent strain of *Vibrio anguillarum*, PT-81049 isolated from a diseased ayu, was used. The cells were cultured on nutrient agar (Eiken Chemical Co. Ltd) at 25 °C for 24 h. Ayu with an average weight of 20 g were immersed in a *V. anguillarum* suspension of 10^6 colony forming units (CFU) ml^{-1} for 5 min. After challenge, the fish were kept in 30 l plastic containers supplied with a constant flow of dechlorinated tap water at 21 °C (± 1 °C). At 1, 3, 6, 12, 18, 24, and 36 to 48 h postchallenge (the period in which dead and dying fish occurred), fish were sacrificed and examined (8 fish at each sampling).

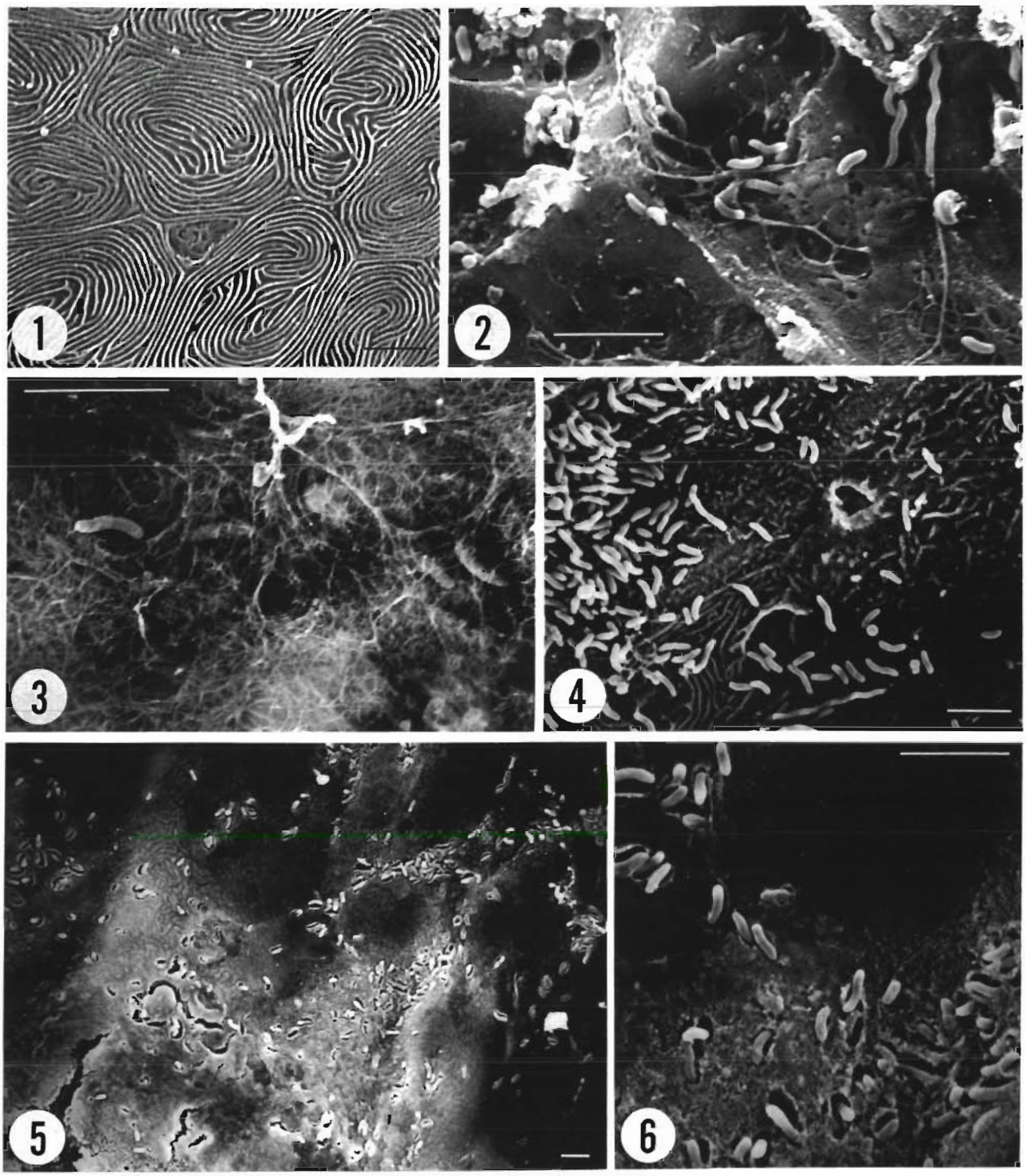
A portion (1 cm^2) of the skin removed from the central trunk was homogenized with sterile saline and inoculated on nutrient agar to determine the number of viable bacterial cells present. For SEM, another portion of the skin was fixed in 1 % glutaraldehyde in cacodylate buffer (pH 7.0) and postfixed in 1 % osmium tetroxide in the same buffer. The samples were sputter-coated with a thin layer of gold and examined with a JEOL JSM-T20 SEM.

Results and discussion. *Vibrio anguillarum* was first detected at 6 h postchallenge at $10^{3.5}$ CFU g^{-1} from the skin. Thereafter, the viable cell number increased rapidly, resulting in $10^{7.8}$ CFU g^{-1} at 24 h postchallenge and $10^{8.8}$ CFU g^{-1} at the moribund stage (36 to 48 h). No significant contamination with other bacteria occurred. The present result differs slightly from that of Muroga & De La Cruz (1987) who failed to detect the pathogen on the skin before 12 h postchallenge and who reported bacterial numbers during advanced stages of the infection that were lower than ours. These differences might be explained by the fact that Muroga & De La Cruz swabbed the skin surface with alcohol-soaked cotton to lessen contamination with water bacteria.

In SEM examinations, no pathological changes or bacterial cells were observed on the skin surface up to 18 h postchallenge. During this period, the fingerprint-like structure of the skin surface remained intact as in the control fish (Fig. 1). By 24 h postchallenge, epidermal sloughing occurred in association with hemorrhagic lesions, and curved short rods were observed in the exposed dermal layer (Figs. 2 and 3). At this stage the fish were still active. By 36 to 48 h postchallenge, dead and dying fish displayed hemorrhages on the skin typical of the disease (Jo 1981). The hemorrhages were extensive, and, at this stage, large numbers of bacterial

* Addressee for correspondence

0177-5103/90/0008/0073/$ 03.00



Figs. 1 to 6. *Plecoglossus altivelis*. Fig. 1. Normal appearance of the skin surface showing its fingerprint-like structure (control); bar = 5 μm. Figs. 2 and 3. Bacterial cells on the hemorrhaging skin (Fig. 2) and in the dermal layer of the hemorrhaging skin (Fig. 3) 24 h after immersion challenge with *Vibrio anguillarum*; bar = 5 μm. Figs. 4 to 6. Large numbers of bacterial cells in the center (Fig. 4) or at the margin (Figs. 5 and 6) of a hemorrhagic skin lesion on a moribund fish (40 h postchallenge); bar = 5 μm

cells appeared on the skin surface through the degenerating epidermis (Figs. 4, 5 and 6).

Muroga & De La Cruz (1987) observed, using the enzyme-labelled antibody technique, that the multiplication of *Vibrio anguillarum* cells occurred in the dermal layer (not on the skin surface, but beneath the epidermis) prior to the pathogen's appearance in any of the other tissues. From these observations they inferred that the pathogen was conveyed to various organs through the blood stream from the sites of proliferation in the dermal layer. The present investigation disclosed that large numbers of the proliferating cells of the pathogen were distributed not only inwards but also to the skin surface. These surface-borne bacteria may account for significant disease transmission between individual fish. Although it was concluded that the primary mode of transmission of vibriosis in ayu is through the water (Kanno et al. 1989), our present results suggest that the disease may also spread rapidly in a crowded pond as a result of direct contact between diseased individuals and noninfected ones.

Responsible Subject Editor: Dr T. Evelyn, Nanaimo, B.C., Canada

LITERATURE CITED

Funahashi, N., Miyazaki, T., Kodera, K., Kubota, S. (1974). Histopathological studies on vibriosis in ayu. Fish Pathol. 8: 136–143

Jo, Y. (1981). Studies on *Vibrio anguillarum* infection in cultured ayu and its immunological prophylaxis. Shikoku Acta Medica 37: 82–110

Kanno, T., Nakai, T., Muroga, K. (1989). Mode of transmission of vibriosis among ayu *Plecoglossus altivelis*. J. aquat. Anim. Health 1. 2–6

Kusuda, R., Sugiyama, A., Kawai, K., Inada, Y., Yoneda, M. (1981). Pathogenicity of *Streptococcus* sp. and *Vibrio anguillarum* in cultured ayu. Bull. Jap. Soc. scient. Fish. 47: 993–997

Muroga, K., De La Cruz, M. C. (1987). Fate and location of *Vibrio anguillarum* in tissues of artificially infected ayu *(Plecoglossus altivelis)*. Fish Pathol. 22: 99–103

Muroga, K., Egusa, S. (1988). Vibriosis of ayu: a review. J. Fac. appl. Biol. Sci., Hiroshima Univ. 27: 1–17

Muroga, K., Yamanoi, H., Hironaka, Y., Yamamoto, S., Tatani, M., Jo, Y., Takahashi, S., Hanada H. (1984). Detection of *Vibrio anguillarum* from wild fingerlings of ayu *Plecoglossus altivelis*. Bull. Jap. Soc. scient. Fish. 50: 591–596

Manuscript first received: August 17, 1989
Revised version accepted: November 28, 1989