RECOVERY OF VIABLE BUT NONCULTURABLE *AEROMONAS HYDROPHILA* CELLS AND MAINTENANCE OF ABILITY TO ADHERE TO MCCOY CELLS AFTER RESUSCITATION

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Abstract

Maintenance of pathogenicity of viable but nonculturable *Aeromonas hydrophila* cells experimentaly stressed at 5°C in natural seawater microcosms was investigated. Pathogenicity, in terms of cytotoxicity and ability to adhere to McCoy cells, was lost concomitantly with culturability, whereas cell viability remained undamaged, as determined by the direct viable count. Recovered cells, by a temperature shift from 5 to 23°C restore their adhesion properties.

Keywords: Aeromonas hydrophila, VBNC, resuscitation, pathogenicity

Aeromonas hydrophila is an opportunist human pathogen which is widely distributed in aquatic environments (1,2). A relationship between changes in water temperature and the incidence of *Aeromonas* spp. has been reported. In seawater within arid regions, aeromonads were found in high numbers in late summer/early autumn when the temperature was around 20-25°C and were rarely detected during cold seasons (3). The inability to isolate *A. hydrophila* during the winter months or from cold waters may result from the entry of cells into the viable but nonculturable (VBNC) state (4). However, contreversial results were produced from attempts to restore culturability. Moreover, in our knowledge, none of the reported studies adressing the pathogenicity of *A. hydrophila* nonculturable and recovered cells.

In this study, *A. hydrophila* ATCC 7966 with cytotoxic activity was used for entering the VBNC state when it was incubated in filtered sterilized natural seawater. Cells were grown on BHI broth, collected and immediately suspended in 0.5-liter bottles containing 100 ml of filter-sterilized natural seawater to obtain a final concentration of 10^8 cfu.ml^{-1} and incubated without shaking at 5°C. At fixed times, samples were collected for culturable, total and Direct Viable Counting (5). After about 45 days of starvation, when the culturable cells declined below the detection level of 0.1 cfu.ml^{-1}, microcosms were shifted to room temperature (23°C) without exogenous nutrient addition. Culturable cells first appeared after one day then increasing to a maximum of 10^4 cfu. ml^{-1} within 3 days of room temperature incubation. Comparison of the growth rates of the stressed population and of the untreated bacteria growing in the same autoclaved initial cell suspension, significantly showed faster growth for the stressed cells, suggesting that in addition to growth of the few culturable stressed cells, a given amount of injured cells entered a culturable state.

In order to test the pathogenicity of culturabe, VBNC, and recovered cells, attachment ability and cytoxic activity with McCoy cells were used. Each bacterial suspension was adjusted to 10^6 viable (i.e. DVC-positive) bacteria and a portion of 300 ?l of a bacterial suspension was added to the cell monolayer. After 1 h of incubation at 37°C in a 5% CO_2 atmosphere to permit bacterial adhesion, the cells were stained with Giemsa for 1 mn and visualized by light microscopy under oil immersion at a magnification of x 100. Our results showed clearly that entry into the VBNC state was accompanied by a loss of the adhesion property (Fig. 1A and B). This loss is transient because, after temperature upshift, the ability to adhere to McCoy cells was recovered. (Fig. 1C).

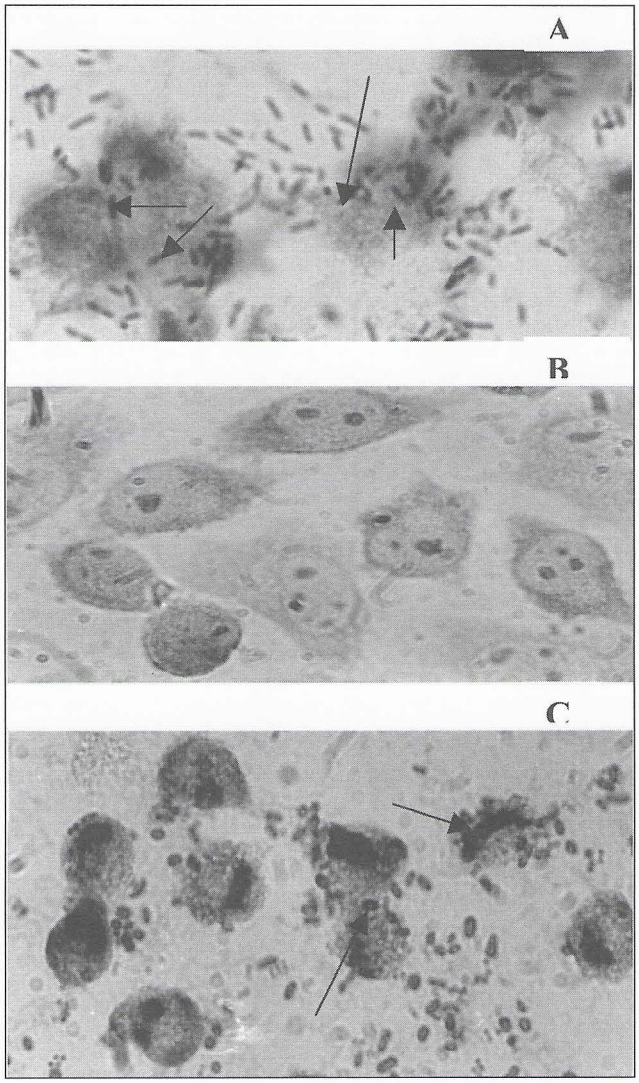

Fig. 1. Photograph of *Aeromonas hydrophila* cells in the adhesion assay with McCoy cells. (A) Adhesion by culturable cells, representing the day 0 sample. (B) Adhesion by VBNC cells collected after 45 days. (C) Adhesion by recovered cells after 3 days.

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