SURVIVAL OF *VIBRIO SPLENDIDUS* BIOTYPE I IN SEA WATER

BY J.E. LOPEZ & L. ANGULO

Vibriosis is one of the most threatening diseases in fish cultures in marine waters. However, vibrioses caused by *Vibrio anguillarum* are the most serious bacterial infections limiting the production of marine fish over the world (Toranzo & Barja, 1990). On the other hand, there are vibrios in the environment which are taxonomically and serologically related to *V. anguillarum* (Fouz *et al.,* 1990) which have recently been associated with fish diseases (Angulo *et al.,* 1992). These vibrios correspond mainly to different biotypes of *V. pelagius* and *V. splendidus,* and can be *designated V anguillarum*-related organisms (Myhr *et al.,* 1991) . The ability of pathogens to survive and remain infective in the environment is considered a major determinant of disease. It was therefore felt to be of interest to determine the survival capacity in filtered sea water of *Vibrio splendidus* biotype I as a measure of the ability of this bacterium to persist and be disseminated in sea water.

Two strains of *Vibrio splendidus* biotype I were used in this study: strain 43N, isolated from diseased turbot *(Scophthalmus maximus)* (Angulo *et al.,* 1994); and strain ATCC 33125 obtained from the American Type Culture Collection. The survival experiments were carried out in tubes containing 20 ml of filtered (0.22µm) sea water taken from Ria de Vigo (Galicia, northwest of Spain). Exponential-phase cultures obtained at 20ºC for 24h in Tryptic soy broth containing 2% NaCl were centrifuged and the resultant pellets resuspended in filtered sea water and ajusted to an absorbance between 0.15 and 0.22 at 610 nm (equivalent to 10^6-10^8 CFU/ml) (Fernandez *et al.,* 1992). Tubes were incubated at 20°C on a rotary

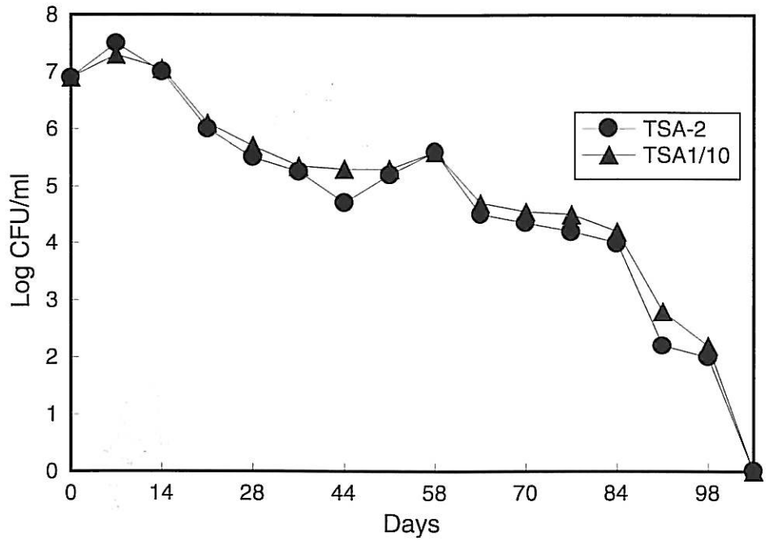

Figure 1. Survival of strain ATCC 33125 in sea water.

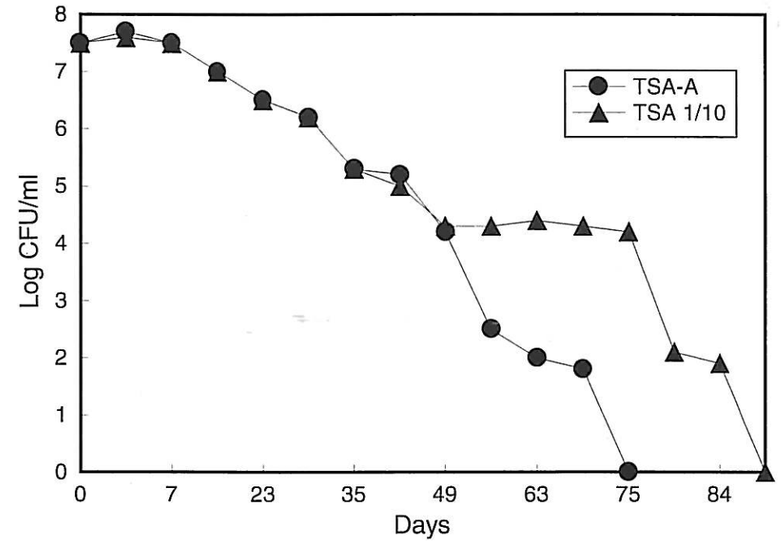

Figure 2. Survival of strain 43N in sea water.

shaker. Periodically, appropriate dilutions of each tube were prepared from zero time to 4 months to enumerate the viable cells on Tryptic soy agar with 2%NaCl added (TSA-2) and TSA 1/10 diluted with distilled water in order to obtain low-nutrient medium. This diluted TSA was supplemented with NaCl to a 2 % final concentration. The survival patterns of the two strains are shown in Figs. 1 and 2. Viable cells of strain ATCC 33125 increased the first 7 days of incubation. Next, the number of bacteria diminished and we were unable to recover culturable cells after 114 days of incubation. No significant differences between the viable cell counts on TSA-2 and TSA 1/10 were observed (Fig. 1). However, strain 43N only survived for 75 days using TSA-2, and survival of this strain was found to be 89 days employing TSA 1/10 (Fig. 2). These results were similar to those of Toranzo et *al.* (1982) who found a survival of *Vibrio anguillarum* of more than 3 months in marine waters. The obtained results indicate that the aquatic environment constitutes a reservoir and a vehicle of transmission of this *Vibrio* species. In addition, we must consider that in natural conditions the physicochemical factors as well as biological factors also play an important role in the survival of this bacterium in marine waters.

Summary

Survival in filtered sea water of two *Vibrio splendidus* biotype I strains was studied. The stability of the two organisms in filtered sea water was only slightly different. Strain ATCC 33125 survived for 114 days on TSA-2 and TSA 1/10. In contrast, strain 43N could not be recovered 75 and 89 days after inoculation using TSA-2 and TSA 1/10, respectively. These results suggest that marine waters are a reservoir of this microorganism.

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