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STUDY OF AEROMONAS HYDROPHILA IN A MARINE ENVIRONMENT : ITS PRESENCE IN SEAWATER, SHELLFISH AND SEDIMENT

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SUMMARY.

The annual distribution of *Aeromonas hydrophila* in seawater, shellfish and sediment was studied, in the marine area affected by the outfall of Guadalhorce river (Málaga. Spain). The results obtained indicate that exists parallel evolution of the average concentrations in the three kinds of sample, and there is a seasonal oscillation with a winter minimum and two maxima in summer and autumn. The results make it possible to consider *A. hydrophila* for sanitary quality of shellfish.

TEXT.

Aeromonas hydrophila is found in all but the most extreme aquatic habitats (1). However, it does not form part of the autochtonous microflora of the sea (2,3), as it happens in fresh water ecosystems. The presence of this microorganism in the sea environments is due to an outside source such as direct discharges of wastewatwer or river outfall.

A. hydrophila is a pathogen of fish and, occasionally, of homeothermic animals, including man. Some epidemiological relationships between the infections produced and the presence of this microorganism in recreational waters are observed. Therefore, it is very important to study the presence of this microorganism in sediments and seawater as well as shellfish, living in those environments, because there are some sanitary hazards for the comsumer population.

The distribution of *A. hydrophila* in seawater, shellfish and sediments was studied in the area affected by the outfall of the Guadalhorce river (Málaga. Spain). Five sample stations, at different depths, were chosen: the depth of two of them is lower than 2 m, the depth of the other two ranges 2 to 5 m and the depth of the fifth is up to 10 m. The sampling was carried out over one year.

The Most Probable Number of *A. hydrophila* per 100 ml or 100 g, was obtained by means of multiple tubes technique (4), using alkaline peptone water (5) as culture medium. The positive tubes were plated on mA agar (6). Identification as *A. hydrophila* of suspected isolates were carried out following the criteria established by Popoff (7).

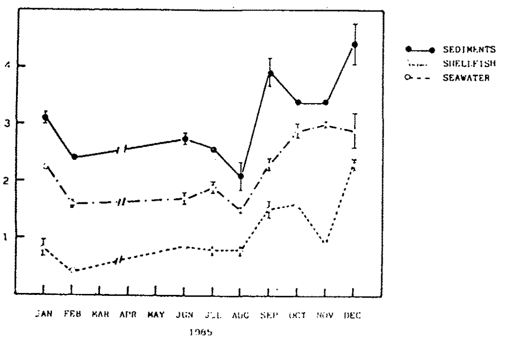
The results indicated in figure 1 show that the annual evolution of *A. hydrophila* concentrations, in the three kinds of samples is almost parallel. The highest levels were obtained in sediments and the lowest in seawater, in agreement with the results of Kaper (2).

On the other hand, the concentrations of this microorganism follow a seasonal oscillation with a winter minimum and two maxima in summer and autumn, with values of 4.50E2/100 ml, 1.10E4/100g and 1.10E5/100 g in seawater, shellfish and sediment, respectively.

The average counts obtained in sediments were 2 or 3 orders of magnitude higher than those obtained in seawater, and 1 order of magnitude higher than those obtained in shellfish.

The high concentrations of *A. hydrophila* observed in shellfish and sediment and the possible relationship with food-borne toxiinfections makes necessary the consideration of this microorganism as an indicator of the sanaitary quality of shellfish.

FIGURE 1. Densities of A. hydrophila by months from sewater, shellfish and sediment.



This work was supported by C.A.I.C.Y.T. grant from Consejería de Educación y Ciencia de la Junta de Andalucía.

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HEAVY METAL RESISTANCE OF ALOCHTONOUS MICROORGANISMS ISOLATED FROM SEAWATER

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SUMMARY.

Resistance of coliforms, fecal streptococci, *Salmonella spp* and *Pseudomonas aeruginosa* strains isolated from coastal waters to the following heavy metals were studied: cadmium, chromium, mercury, lead and zinc. The results obtained show that the highest degrees of resistance to the mentioned metals appear for *P. aeruginosa* strains and the lowest ones for fecal streptococci and *Salmonella spp.*

TEXT.

The increase of heavy metal resistant strains, isolated from different environments contaminated by metals, suggest a selective pressure of these contaminants on the microorganisms (1,2,3). Jones and Cobet (4) propose metallic ions as an important factors in the inactivation of microorganisms in the marine environment. Transferable metal resistance and resistant strains selection could be the main causes of the higher isolation frequency of heavy metal resistant strains in the marine environment.

We studied the heavy metal resistance patterns in seawater isolates of coliforms, fecal streptococci, *Salmonella spp* and *Pseudomonas aeruginosa*. The samples were obtained on coastal waters near Guadalhorce river mouth and on sewage-polluted beaches in Málaga (Spain). The isolation of coliforms, fecal streptococci and *P. aeruginosa* was made by the membrane filtration technique (5) on m-Endo broth, m-Enterococcus agar (5) and mPA-D agar (6), respectively; and by the method proposed by Moriñigo et al. (7) for *Salmonella spp* . Heavy metal susceptibility tests were carried out according to the agar dilution method (8).

The results obtained are summarised in table 1, where the concentration of each metal that inhibits a 90% of strains of each group is shown. *P. aeruginosa* has a higher resistance level to metals than other microorganisms, with minimal inhibitory concentrations about 4 to 16 fold higher, according to the metal and microorganism considered.

All the microorganisms presented the same minimum inhibitory concentration (M.I.C.) to lead. The tolerance profiles for the other four metals are different between the several groups, these MIC ranges are relatively extensive in most of the cases. *Salmonella spp* and fecal streptococci have very similar resistance patterns, resisting the same concentrations of cadmium and mercury. *P. aeruginosa* and coliforms resist higher concentrations of all metals than the other two groups show, finding the highest in *P. aeruginosa*.

Only the MIC to mercury presented a bimodal distribution, with two peaks, being 2 and 64 µg/ml for *P. aeruginosa*, 1 and 8 for coliforms and 1 and 4 for *Salmonella spp.*

In general, a heavy metal resistance scale of the studied microorganisms could be esteblished in the following way: *P. aeruginosa* > coliforms > fecal streptococci ≃ *Salmonella spp.*

The detected MIC values are always higher than the metal concentrations in the water samples studied. Thus, we can suppose that the metals do not exert a selective pressure on the microorganisms considered in this area.

TABLE 1. Metal inhibitory concentrations for more than 90% of strains belonging to each microorganism group (µg/ml).

METAL (Assayed salt)	MICROORGANISMS			
	Coliforms	Fecal Strept.	*Salmonella spp*	*P. aeruginosa*
Cadmium ($CdCl_2$)	400	200	200	1600
Chromium (K_2CrO_4)	400	200	400	3200
Mercury ($HgCl_2$)	16	4	4	64
Lead ($Pb(CH_3\text{-}COO)_2$)	3200	3200	3200	3200
Zinc ($ZnSO_4$)	1600	3200	800	3200

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