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Introduction

Staphylococcus aureus and *Candida albicans* are very widespread microorganisms which have been isolated from aquatic environments (BUCK & BUBUCIS, 1978; BORREGO *et al.*, 1987). The presence of these microorganisms in waters possesses a health hazard since they can infect ears, eyes, cuts, scratches on the skin of bathers.

In this paper, the effects of seawater on the survival of *S aureus* and *C. albicans* compared to the survival of two indicator microorganisms were studied under controlled diffusion chamber conditions.

Materials and Methods

The chambers used in this study were designed as described by FLIERMANS & GORDEN (1977), and before use they were autoclaved for 20 min at 121°C. UV-sterilized polycarbonate filters of 90 mm diameter and 0.2 m pore size (Nucleopore) were used as side walls for the chambers. The chambers were filled with filtered seawater, and transported to the experimentation area in containers filled with seawater. The strains employed in this study were: *Escherichia coli* (two strains isolated from seawater), *Enterococcus faecalis* (ATCC 19933 and one isolated from seawater), *S. aureus* (ATCC 29213 and one isolated from seawater) and *C. albicans* (CECT 1394). In the sampling area, a sterile syringe was used to inoculate 1 ml of the final cell suspension (c.a. 108 cfu/ml) of each strain into each chamber.

Enumeration of the samples was carried out following two procedures, the double agar-layer technique (ANDERSON *et al.*, 1983) used as control medium, and plating directly on selective agar plates. Tryptic Soy Agar (TSA) (Difco) was used as reference medium, and as bottom agar layer. The selective media used were: Endo Agar (Difco) for *E. coli*, KF Agar (Merck) for *E. faecalis*, Mannitol Salt Agar (Difco) for *S. aureus* and mCA Agar (BUCK & BUBUCIS, 1978) for C. albicans. All samples were incubated at 37°C for 24 h. The percentages of both surviving and injured cells during time t were calculated by means of equations (1) and (2):

(1) % Survival = (Count on TSA at time t/Count on TSA at time 0) x 100

(2) % Injury = [l-(Count on selective media at time t/Count on TSA at time t)] x 100

Microbial inactivation was evaluated by applying the law of logarithmic decrease :

$C = CoxlO^{-t/T90}$ where, Co and C are the initial microbial concentration and the microbial concentration at t time, respectively, measured in hours. T90 is a constant characteristic of the process, that represents the time in which the initial population is reduced by 90 %.

Results and Discussion

In Table 1 it can be observed that the survival percentages for all the microorganisms tested were lower than 10 %. The highest percentages of injured cells at the end of the experiments (48 h of immersion) corresponded to *S. aureus* and *E. faecalis*, and T90 values of these microorganisms were also very similar. The survival of *C. albicans* was very similar to those of indicator microorganisms, and even at the end of the experiments the population of *C. albicans* showed a lower injury percentage than the other microorganisms. In contrast, the T90 value for *Candida* is the lowest. These data do not agree with those results which reported similar survival rates of *C. albicans* compared to indicator and other pathogenic microorganisms (CORNAX *et al.*, 1990). This result may be explained by a higher sensibility of this microorganism to the experimental manipulation before introduction into the diffusion chamber (CORNAX, 1986). This fact could provoke a rapid death of the injured cells, but this reduction of the population would not be due to the effect of seawater.

The lower injury percentages of *E. coli* and *C. albicans* in seawater may be due to the fact that they show different abilities to use the organic nutrients present in seawater. Similarly, SINCLAIR & ALEXANDER (1984) demonstrated that *E. faecalis* had a low capability to obtain energy from natural waters. However, several studies pointed out that the lack of nutrients in the water do not seem to be the main factor affecting the inactivation process of microorganisms in seawater (FUJIOKA *et al.*, 1981; DE VICENTE*et al.*, 1988; MORINIGO *et al.*, 1989; CORNAX *et al.*, 1990).

Table 1. Injury and survival rates and T90 values of the microorganisms in diffusion chambers experiments

Microorganisms	Injury percentage	Survival percentage	T90 value (hours)
E. coli	83.3 ± 4.6	8.0 ± 1.6	47.2
E. faecalis	94.0 ± 2.7	7.3 ± 3.6	37.0
S. aureus	96.2 ± 3.6	6.0 ± 2.9	34.0
C. albicans	79.4 ± 2.8	8.0 ± 3.3	24.0

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