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Competitive Elimination of *Enterobacteriaceae* from Seawater[1]

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The more or less rapid elimination of *Escherichia coli* from seawater represents an extremely complex phenomenon. Bactericidal or lethal effects were studied with regard to the presence of heat-labile substances (7, 11), heavy metal ions (7), bacteriophages (2), and cell wall degrading or lysing microorganisms (8). In addition, it is generally assumed that the survival of microorganisms of nonmarine origin in seawater is basically affected by the mere competition for growth-limiting nutrients, primarily carbon and energy sources (1). During recent studies on the growth kinetics of various marine isolates, data were obtained on the basis of which the role of growth competition, as compared to more specific inhibitory effects, could be evaluated and defined.

A chemostat inoculated with a mixed population will select for that organism that exhibits the fastest growth rate under the specific conditions (composition of medium, temperature, etc.). These competitive processes were studied in detail (4) with sterile supplemented seawater as a medium and raw seawater as an inoculum. A number of bacterial strains belonging to different genera were enriched by varying the dilution rate or the concentration of the growth limiting substrate in the reservoir, or both. In these experiments, 0.1 to 10 mg/liter of lactate, glycerol, or glucose were added to filter-sterilized offshore seawater; ammonium and phosphate were added in sufficient concentrations to ascertain growth limitation by the carbon and energy source. Samples taken from the chemostat twice a retention time were streaked on agar that was prepared with the same medium but contained 100 mg/liter of the respective carbon source. Enrichments to at least 90% of one colony type or individual strain were completed in periods of 5 to 10 retention times, or 15 to 84 hr.

The criteria for successful competition (enrichment) or unsuccessful competition (displacement) of a particular species can be expressed in terms of growth parameters: the maximum growth rate (μ_m) at excess concentration of the limiting substrate, and the substrate saturation constant (K_s), which equals that concentration of the limiting substrate giving rise to half of the maximum growth rate (3, 9). Species exhibiting relatively low K_s and μ_m values will displace species with relatively high growth parameters if the continuous culture is run at correspondingly low dilution rates or low concentrations of the limiting substrate in the reservoir, or both (4).

During a survey-type study with supplemented seawater, a variety of successful competitors were isolated in pure culture. Table 1 shows the results of such an experiment with a dock-water sample as the inoculum; the isolates were arranged according to the particular conditions of their successful enrichment. From this inoculum, some strains were isolated which later could be identified as belonging to the *Enterobacteriaceae*. The position of *Aerobacter* spp. and *Escherichia coli* in Table 1 indicates their ability to overgrow the rest of the isolates at relatively high dilution rates and relatively high concentrations of the limiting substrate. These results demonstrate the highly selective effect inherent to counting or isolation techniques that employ agar or liquid media with nutrient concentrations higher than about 0.05%. The genus determinations in the present study were made with substrains on media of the usual strength.

The puzzle of frequent occurrence of *E. coli* in mid-ocean areas and at considerable depth was solved when a sampler was used that absolutely prevented contaminations from the sample gear passing through the surface film of the seawater surrounding the research vessel on station (6).

The absolute values of the growth parameters given in Table 2 allow the prediction of enrichments or of the rapidity of the separation of two species from mixed pure cultures under the given conditions. Thus, it was possible to select for either one of two competitors by the choice of the critical dilution rate or concentration of the limiting substrate (4). Figure 1 shows the ranges of successful competition for *Spirillum* spp. (strain 101) and *E. coli* (strain 415).

It has been noted that growth parameters might

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TABLE 1. *Continuous culture enrichments of various bacteria from inshore seawater in 16 experiments that varied the dilution rate and the concentration of the limiting substrate in the reservoir.*[a]

Concn of lactate in the reservoir (mg/liter)	Dilution rate ($hour^{-1}$)			
	0.05	0.10	0.25	0.50
100.0	A, C	*Pseudomonas*, C	*E. coli*, C	*E. coli*, C
10.0	A, C	*Pseudomonas*, C	*Aerobacter*	*Aerobacter*
1.0	*Achromobacter*	*Vibrio*	*Pseudomonas*, B	*Aerobacter*
0.1	A	*Achromobacter*	A, B	A, B

[a] A, no appreciable enrichment within eight retention times; B, heavy wall growth; C, turbidity.

TABLE 2. *Growth parameters as obtained from continuous or batch culture in filter-sterilized offshore seawater at 20 C for two limiting substrates: K_s in mg/liter, μ_m in hr^{-1}*

Culture	Lactate		Glucose	
	K_s	μ_m	K_s	μ_m
Vibrio spp. (strain 204)	0.8	0.15	5.5	0.40
Achromobacter spp. (strain 208)	1.0	0.15	3.0	0.35
Spirillum spp. (strain 101)	3.0	0.45		
Aerobacter spp. (strain 417)	6.0	0.50	5.0	0.45
Escherichia coli (strain 415)	9.0	0.80	8.0	0.65
Serratia marinorubra	15.0	1.10		

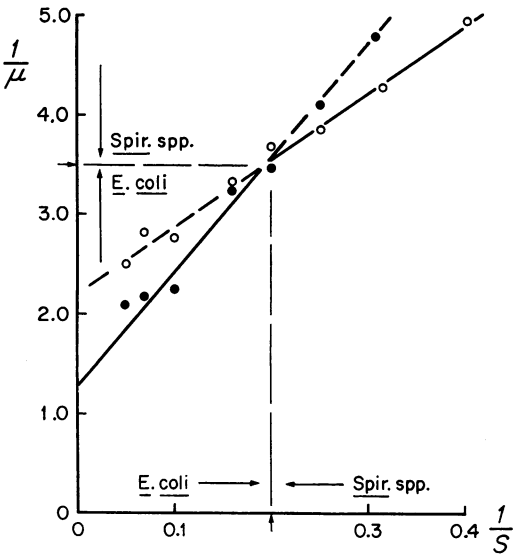

FIG. 1. *Reciprocal plot of concentration of limiting substrate (S, in mg of lactate/liter) versus growth rate (μ, in $hours^{-1}$), demonstrating the ranges of successful competition (solid line) between Spirillum spp. strain 101 (○) and Escherichia coli, strain 415 (●).*

increase if the organisms are grown for a prolonged period of time on media of higher strength than that used for the enrichment (5). Therefore, the growth parameters of a stock culture strain of *Serratia marinorubra* appear extremely high. Reducing growth parameters experimentally by transferring a washed cell suspension into a batch culture containing no, or relatively little, substrate concentration (10^{-6} M of the carbon source) has proven unsuccessful so far. Under such conditions it was possible, however, to store cultures for more than 6 months without an increase of growth parameters. During this period, the cell count dropped to about 25% of the initial count. If an initial concentration of 10^{-4} M of the carbon source was applied, no survival was detected after 2 months. This effect may be related to the phenomenon of "substrate accelerated death" described by Postgate and Hunter (10).

According to the growth parameters given in Table 2, the competition in the natural environment will be decided by the product of factors equivalent to the experimental dilution rate, and by the natural concentrations of limiting substrates. Both values are unknown and, undoubtedly, variable. If, however, a more or less indiscriminating elimination of microbial cells in seawater is assumed (for instance, by predation), an organism competing unsuccessfully for a growth-limiting substrate will be displaced eventually.

In order to assess natural growth rates, nonsupplemented filter-sterilized seawater from various off- and inshore areas was run through the chemostat inoculated with raw seawater or pure cultures. In all cases, bacterial numbers decreased nearing complete outwash even at dilution rates as low as 0.012 hr^{-1}. In other words, no organism was able to maintain a suspended population in unsupplemented seawater at experimentally imposed doubling times as high as 84 hr. When these experiments were done with a variety of inshore waters, the decrease of bacterial numbers was distinctly slower than the dilution rate, indicating some growth.

This indication of extremely low in situ growth rates demonstrates, in connection with the values given in Table 2, that *Enterobacteriaceae* have little or no chance to outgrow competitors in seawater. The data substantiate the assumption that competitive displacement of *Enterobacteriaceae* from seawater under natural conditions represents a phenomenon underlying other effects of direct bactericidal action.

ACKNOWLEDGMENT

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