JOURNAL OF BACTERIOLOGY, Feb. 1968, p. 722-723

Vol. 95, No. 2
Printed in U.S.A.

Growth Characteristics of Heterotrophic Bacteria in Seawater[1]

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Received for publication 18 November 1967

Studying *in situ* rates of microbial transformations of dissolved organic materials in the sea is impeded by the minute concentrations of substrates present and the complexity of seawater as the medium for analytical work. As an indirect approach, growth studies in continuous culture have been used successfully and have led to some results of general interest.

When a chemostat was fed with sterile-filtered and supplemented seawater and inoculated with a nonsterile seawater sample, the resulting selective conditions (dependent upon dilution rate and concentration of the limiting substrate) permitted a separation and isolation of species of different growth characteristics. The seawater in the reservoir was supplemented with sodium lactate or glucose in quantities from 10^{-6} to 10^{-3} M, while ammonium and phosphate were added in amounts to ascertain growth limitation by the carbon and energy source. The details of these enrichment experiments are published elsewhere (H. W. Jannasch, Arch. Mikrobiol. **59**:165, 1967).

At low concentrations of the limiting substrate in the reservoir (10^{-6} to 1.5×10^{-4} M), streaking of the mixed culture, at intervals of half a retention time, on agar medium containing the corresponding carbon source (10^{-2} M) showed an increase of small colonies, whereas species producing considerably larger colonies were enriched in the presence of higher substrate concentrations. Subsequent pure culture studies of the isolates resulted in a list of species which differ markedly in their growth constants (Table 1A). The maximal growth rate (μ_m) was measured either in batch culture or calculated from outwash rates in continuous culture. The substrate saturation constant (K_s) was calculated from enzymatic determinations of substrate concentrations during steady state by using Monod's original equation (J. Monod, *Recherches sur la Croissance des Cultures Bactériennes*, Hermann, Paris, 1942).

The constants reported in Table 1A increased considerably during continued transfers on agar media containing 10^{-2} M of the limiting substrate (Table 1B). No appreciable changes were found when the chemostat cultures were membrane-filtered, rinsed, and resuspended in sterile filtered seawater. In such a suspension, 10 to 25% of the initial cell count was recovered after 8 months of storage at 18 C. In all tested cases, a loss of the original growth characteristics was also found when the organisms were grown in a peptone-yeast extract medium of 0.5% each. The genus determinations given in Table 1 have been done with substrains adapted to higher substrate concentrations.

The data suggest that part of the heterotrophic marine bacterial population is adapted to growth on the low substrate concentrations present in seawater and able to compete successfully with species growing less efficiently under the given conditions. Organisms of the latter group may occur either as dormant stages or, as often suggested, associated with particles supplying nutrient concentrations higher than the surrounding seawater. This may be interpreted as reviving

TABLE 1. *Growth constants[a] of several marine isolates for two limiting substrates, measured at 20 C before (A) and after (B) growth on 10^{-2} M of the corresponding substrate for at least five transfers[b]*

Isolate	Constant	A		B	
		Lactate	Glucose	Lactate	Glucose
Achromobacter sp. (strain 208)	K_s	1.0	3.0	5.0	12.0
	μ_m	0.15	0.35	0.40	0.90
Vibrio sp. (strain 204)	K_s	0.8	5.5	9.5	—
	μ_m	0.15	0.40	0.80	—
Spirillum sp. (strain 101)	K_s	3.0	—	12.0	—
	μ_m	0.45	—	1.10	—
Pseudomonas sp. (strain 201)	K_s	9.0	10.5	15.0	18.0
	μ_m	0.80	0.95	1.40	1.60

[a] Constants: K_2 in 10^{-5} M; μ_m in hours^{-1}.
[b] Maximum error for K_s values, 12.5%; for μ_m values, 6.4%.

[1] Contribution no. 2021 from the Woods Hole Oceanographic Institution.

Winogradsky's early concept of "autochtonous" and "zymogenous" parts of a microflora. It also implies that solid and liquid media of the usual strength and in closed systems will fail to yield successful isolations of indigenous species actively engaged in the decomposition or transformation of dissolved organic carbon in seawater.

Another observation showed that in continuous culture of some of the isolates the limiting substrate was not consumed beyond a certain threshold concentration. It is generally accepted [e.g., D. Herbert, Soc. Chem. Ind. (London) Monograph **12**:21, 1961] that, during steady state, the population density is dependent upon the concentration of the limiting substrate in the reservoir and independent of the substrate concentration in the chemostat. In seawater, this was often found to be true only at high population densities. Upon decreasing the substrate concentration in the reservoir below a certain threshold value, premature outwash of the culture occurred (H. W. Jannasch, Limnol. Oceanog. **12**:264, 1967). This concentration of the limiting substrate corresponded to a minimal population density below which, at a given dilution rate, the population was unable to maintain itself in the chemostat.

An explanation of a similar phenomenon has been given for the case where growth of a microaerophilic bacterium was inhibited by constant aeration only at low population densities (H. W. Jannasch, Arch. Mikrobiol. **45**:323, 1963). In other words, if the metabolic activity of the population affects the environmental conditions other than by lowering the concentration of the limiting substrate, growth limitation in continuous culture may become a function of population density. The inhibitory character of this effect, as opposed to effects of endogenous respiration or maintenance metabolism, is indicated by an increase of the substrate concentration in the chemostat (Fig. 1).

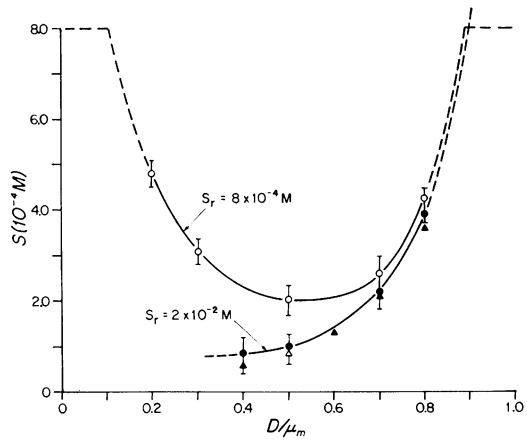

FIG. 1. *Steady-state concentration of lactate (S) in the chemostat of Pseudomonas sp. (strain 201) versus relative dilution rate (D/μ_m) at two different concentrations of lactate in the reservoir (S_r), and the ranges of standard deviation. Triangulars indicate the theoretical values ($\Delta = K_s$).*

Novick's observation (Ann. Rev. Microbiol. **9**:97, 1955) that *Escherichia coli* was washed out from the chemostat after being "forced into lag" at extremely low dilution rates (0.06 hr^{-1}) is unlikely to correspond with the effect observed here at dilution rates of 0.64 to 0.16 hr^{-1}. No deviation of the population density data from the theoretical values has been found at S_r values higher than 10^{-2} M lactate within the range of growth rates applied.

Thus, the observed phenomenon seems to represent an expression of the suboptimal character of seawater as a base medium for growth of some heterotrophic bacteria.

This study was supported by National Science Foundation grant GB 5199.