Vol. 53: 101–103, 1989 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published April 4

NOTE

Microbial activity in water overlying the nearshore sand substratum in Natal, South Africa

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ABSTRACT: Small, free-living bacteria have been considered to be either dormant or heterotrophically active and adapted for efficient survival on low concentrations of UDOM (utilisable dissolved organic material) by way of starvation-survival. They were, in fact, shown to be heterotrophically active in the water column of a subtidal reef in Natal (South Africa), an environment containing UDOM derived from accumulated plant litter. They are more prevalent over the sand substratum between such reefs, where plant litter is transient and the concentration of organic matter (5.4 mg l^{-1} TOC) is only 30 % of that found on the reef studied. Simple uptake experiments conducted in both summer and winter, using a labelled algal extract (LAE) as substrate, showed the bacteria over the sand to be as active as those over the reef. In most cases, uptake of LAE commenced immediately after addition and progressed linearly; short-term uptake site saturation possibly occurred in the few remaining cases. Results appear to conform to the starvation-survival theory.

The abundance of small, free-living bacteria in the aquatic environment is well documented, recent noteworthy reviews on their role in the marine environment being those of Azam et al. (1983) and Lucas (1986). In some earlier work it was suggested that these bacteria were dormant (Wangersky 1977, Stevenson 1978), but other research clearly showed that they account for considerable heterotrophic activity in many environments (Williams 1970, 1981, Berman 1975, Azam & Hodson 1977, Cole & Likens 1979, Fuhrman 1981, Schleyer 1981).

Extensive work of such a nature was carried out on a nearshore, subtropical, subtidal reef in Natal, South Africa (Schleyer 1980, 1981, 1984). These studies supported the latter finding and were later extended to the expansive and less productive sand substratum interspersing Natal's reef systems (Schleyer & Roberts unpubl.). Due to water exchange, the bacterial populations are common to both environments and were found to be almost uniform in number (2×10^6 ml^{-1}), community structure and heterotrophic activity (mean V_{max} values of 293 mg C m^{-3} d^{-1} and 244 mg C m^{-3} d^{-1} were obtained over the reef and sand respectively; Schleyer 1984, Schleyer & Roberts unpubl.). The only major difference between the populations is a proportionately greater number of free-living bacteria over the sand (92.8 %; Schleyer & Roberts unpubl.) than over the reefs (79.0 %; Schleyer 1981). However, the populations are spatially and temporally well separated and are distinct in terms of their food availability; bacteria on the reefs have available a rich source of fine particulate and dissolved organic carbon (FPOC and DOC), derived mainly from accumulated plant detritus (Schleyer 1981), in contrast to the sand substratum over which plant litter is merely transient in its passage. Total organic carbon (TOC) levels of only 30 % ($\bar{x}$ = 5.4 mg l^{-1}) of those found on the experimental reef were measured over the sand. The sand environment is thus nutrient-depleted compared to that of the reefs and it was suspected that the 'deprived' bacteria may manifest dormancy.

For this reason it was decided that the free-living bacteria over the sand substratum provided a good opportunity to conduct a simple test for dormancy. It had already been established that the incubation of samples with tracer quantities of a labelled algal extract (LAE) elicited 'normal' rates of uptake over a 1 h period (Schleyer & Roberts unpubl.), so the question posed was whether an induction period was required before uptake commenced. The linearity of progressive uptake of LAE was thus monitored for this purpose.

Methods. In 2 separate experiments, conducted in summer and winter, water samples were collected in the surf and backline at beaches off Addington and Sunkist in Durban, South Africa. The former site is roughly 200 m from the nearest reef and is fairly sheltered from wave action by the Durban Harbour breakwaters. The latter is 2.5 km from a groyne, the nearest reef-like feature, and is an exposed, high-energy

0171-8630/89/0053/0101/$ 03.00

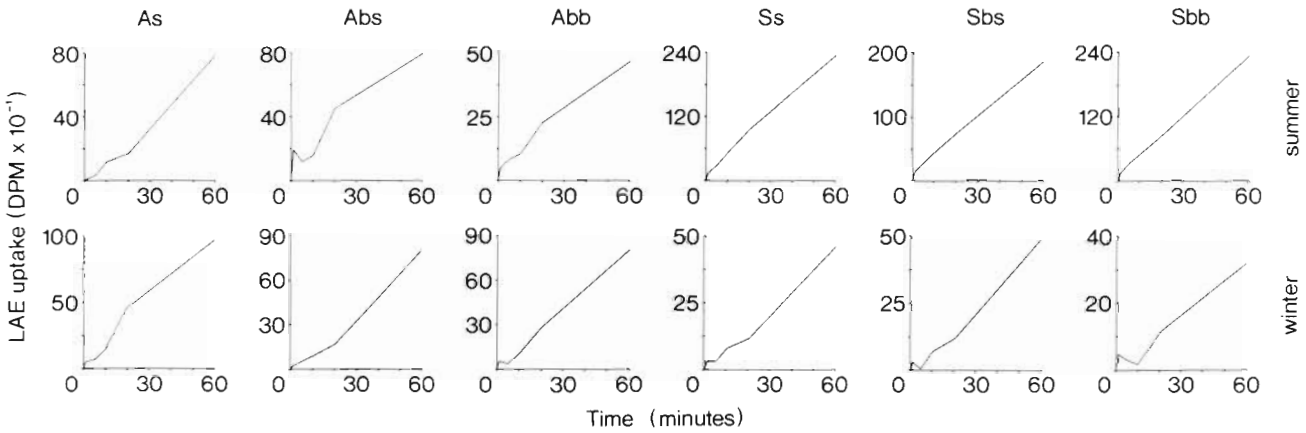

Fig. 1. Progressive uptake of LAE (corrected DPM) in water samples collected at Addington (A) and Sunkist (S). Samples were collected in the surf (s) and backline at the surface (bs) and bottom (bb). Experiments were performed at 25 °C (summer) and 20 °C (winter)

beach. Water temperature was measured at the sampling sites and the samples were collected in sterilised, 1 l amber glass reagent bottles. This was done at the surface and bottom (4 to 6 m depth) at the backline, and midwater in the well-mixed 0.5 to 1.0 m deep surf.

The samples were immediately transported to the laboratory in an insulated container and processed as follows. Aliquots of 10 ml were drawn into sterile, evacuated, rubber-capped glass tubes (commercially available for blood collection). These were injected with LAE of specific activity 58.07 μ Ci mg^{-1}, the preparation of which was described by Schleyer (1984), to a final concentration of 21.5 μ mg l^{-1}. Samples were incubated for 1, 5, 10, 20 or 60 min in the dark at 25 °C (summer) or 20 °C (winter). Controls consisted of sterile-filtered seawater with LAE added to provide background counts and test the sterility of the LAE. At the end of incubation, particulate matter in each sample was then digested by 100 μl phenylethylamine in a scintillation vial. Instagel (10 ml) was added and the radioactivity was counted in a suitably calibrated scintillation counter. Progessive uptake in corrected DPM was finally plotted as a function of time after subtraction of the invariably low control counts.

Results. Experimental results are presented in Fig. 1. Uptake of LAE appeared to commence immediately after addition and to progress continuously in most cases, at both summer and winter temperatures. In the few instances in which uptake was briefly reduced after the first few minutes of heterotrophic activity, short-term uptake-site saturation may have occurred. There was no evidence of the need for an induction period which one would expect to encounter in dormant bacteria before transition to an active state. In fact, uptake in the first minute was often the most rapid.

Discussion. The small size of free-living aquatic bacteria appears to be associated with an adaptation known as 'starvation-survival', a subject reviewed by Morita (1982; see also MacDonell & Hood 1982, Azam & Ammerman 1984, Schleyer 1986). Natural utilisable dissolved organic material (UDOM) normally occurs in the environment at low concentrations and the free-living bacteria use this resource at maximal efficiency by this process. It is characterised by a reduction in size of the bacteria, an increase in their number, and the expenditure of reserve material on the production of ATP, RNA and specific proteins such as uptake enzymes. The process is further enhanced by the fact that, as most aquatic bacteria are gram-negative, their hydrolytic and binding enzymes are contained in a periplasmic space just inside the cell wall. Their small size also endows them with a high surface-to-volume ratio, and they thus possess a proportionately large and efficient absorptive area for the immediate uptake and utilization of substrates at low concentrations. Bell & Gutstein (1981) have further demonstrated that they possess a diversity of enzyme systems, which permits them to use UDOM from a variety of sources.

Bacteria over the sand showed no sign of dormancy, despite the reduced availability of food, and they manifested activity which would be consistent with the starvation-survival theory. The short-term uptake site saturation which was apparent in some samples does not detract from such a suggestion; immediate uptake of the LAE did occur in these samples and the microbes were merely unable to cope with the concentration of added nutrition in the short-term.

Acknowledgements. Financial support for this programme by the South African National Committee for Oceanographic Research and the South African Association for Marine Biological Research is gratefully acknowledged.

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Manuscript first received: August 5, 1988
Revised version accepted: January 10, 1989