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Anaerobic starvation survival of marine bacteria

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Abstract

Starvation affects marine bacteria also under anaerobic conditions. Some basic data obtained for anaerobic starvation survival of a fermentative and a sulfate-respiring strain indicate substantial differences. The fermentative strain, *Listonella (= Vibrio) anguillarum*, responded to nutrient depletion with rapid reduction of their cell size (dwarfing) and decline of viable cell counts by three orders of magnitude. The sulfate-respiring *Desulfovibrio vulgaris* showed only minor reductions of the cell sizes and no loss of viability. Whereas a drastic decline of cellular protein concentrations in this strain indicated strong endogeneous respiration, starved cells of the fermenting *Vibrio* sp. showed increasing levels of protein after an initial decrease.

Introduction

Concentrations of dissolved organic carbon in the marine environment are often below 0.8 mg/l (THURMAN 1986). This puts copiotrophic bacteria, which represent the rapidly cultivated constituents of the marine microflora, frequently to a famine existence.

During the initial phase of starvation the number of living cells changes rapidly, until a fairly constant cell density is reached. This response is paired with "dwarfing" of the cells (rounding up and decreasing in cell volume - AMY and MORITA 1983). Nutrient depletion induces an energy-dependent reorganisation of cell constituents such as "protein-fingerprints" (JAAN et al. 1986), and leads to increasing substrate affinities, degradation of storage compounds, enhanced endogenous respiration (KJELLEBERG et al. 1987) and fatty-acid profile changes (GUCKERT et al. 1986) affecting the hydrophobicity of the cells (KJELLEBERG and HERMANSSON 1984).

Despite the abundance of facultatively anaerobic copiotrophs such as *Vibrio* spp. in the marine environment, not much attention (SMIGIELSKI et al. 1990) has been paid to anaerobic starvation which can be expected in reduced sediments including numerous microhabitats such as the gut of marine animals. Some basic data on two strains representing fermentative and respiratory anaerobes reveal quite different responses to anaerobic starvation.

Material and methods

Organisms: *Listonella (= Vibrio) anguillarum* isolated from the internal fluid of a bivalve (*Arctica islandica)* was grown in liquid culture with 0.1 % yeast-extract, 0.5 % peptone and 1.5 % NaCl. *Desulfovibrio vulgaris* (DSM 1744) was grown in brackish water medium with lactate for sulfate-reducing bacteria (WIDDEL 1980).

Starvation survival experiments: Cultures were harvested at the end of their exponential growth phase and washed 3 times with mineral medium (MMS). *Listonella* sp. was starved in modified salt medium (MMS: NOVITSKY and MORITA 1978) reduced with dithionite. *D. vulgaris* was starved in basal medium for sulfate reducers without organic substrate (WIDDEL 1980).

Total direct counts and cell volumes were determined by acridine orange epifluorescence microscopy (HOBBIE et al. 1977). Cell volumes were calculated as median values of n = 100 size measurements using a "New Porton" grid G12.

Total viable direct counts were obtained after incubating non-fixed samples of *L. anguillarum* and *D. vulgaris*, for 8 h and 24 h, respectively, with 0.002 % nalidixic acid and 1 g/l of yeast extract or 15 mM of lactate (KOGURE et al. 1979, modified).

Protein in washed cell suspensions was determined according to LOWRY et al. (1951).

Results and discussion

Anaerobic starvation of *Listonella anguillarum* caused a rapid decrease of viable cells during the first 200 h with only little change afterwards, whereas viability of *Desulfovibrio vulgaris* showed no decline (Fig. 1).

Within the first week of starvation, cell volumes of *L. anguillarum* declined to about 12 % of their initial size. During the same length of time, cell volumes of *D. vulgaris* were reduced by only 5 % (Fig. 2).

Starvation of *D. vulgaris* led to a sharp decrease of protein concentrations per cell volume. On the other hand, *L. anguillarum* showed a starvation dependent increase of these protein concentrations (Fig. 3).

Under anaerobic conditions, starvation survival of the facultative anaerobe, *L. anguillarum*, appears considerably less efficient than in the case of the obligate anaerobe, *D. vulgaris*. Size reduction of the cells and their transformation to coccoid forms seem to occur independently of the ambient redox potential.

The remarkable maintenance of viability of the sulfate-reducing *D. vulgaris* is presumably made possible at the expense of endogenous respiration of cellular proteins. Considering the rather specific substrate requirements of sulfate reducers (WIDDEL 1980), these obligate anaerobes are likely to incur frequently periods of nutrient depletion in their environment.

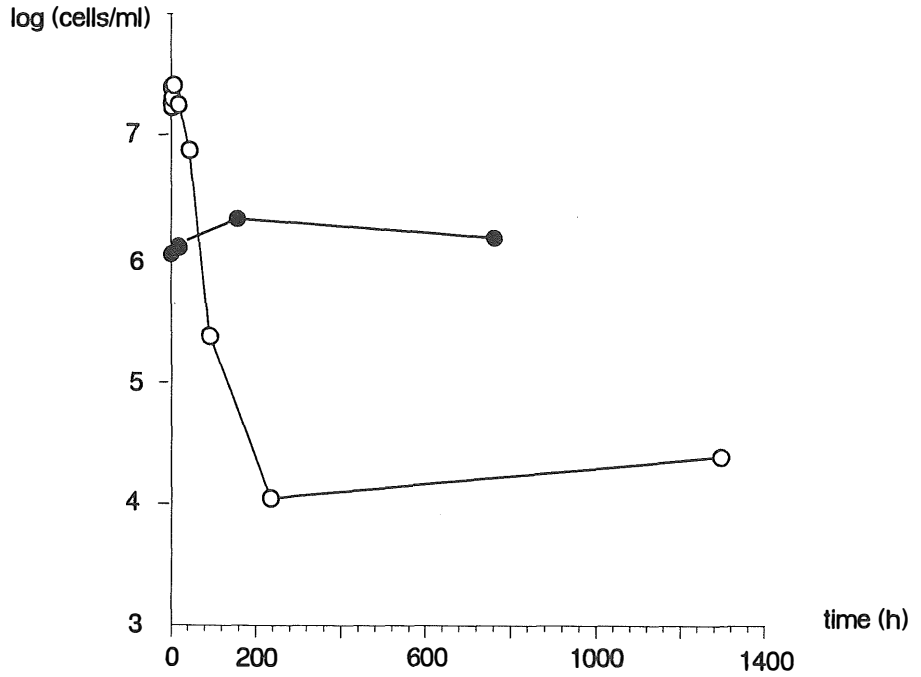

Fig. 1. Viable counts of *Listonella anguillarum* (o) and *Desulfovibrio vulgaris* (●) under anaerobic starvation.

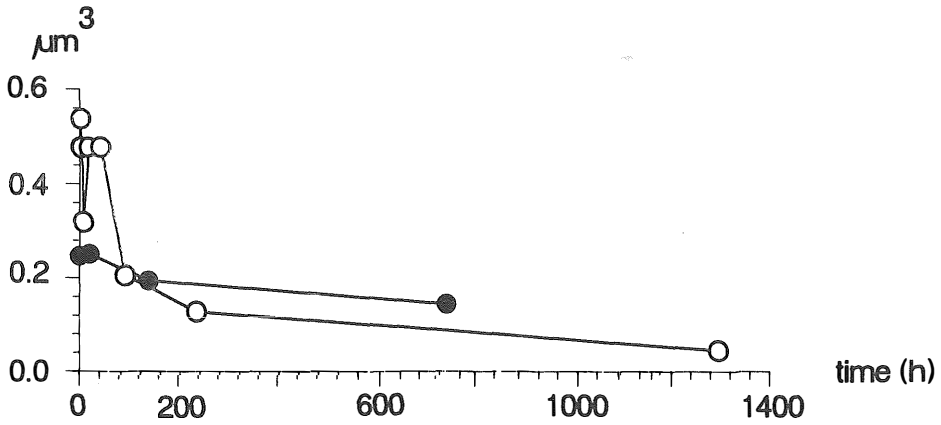

Fig. 2. Cell volumes of *L. anguillarum* (o) and *D. vulgaris* (●) under anaerobic starvation.

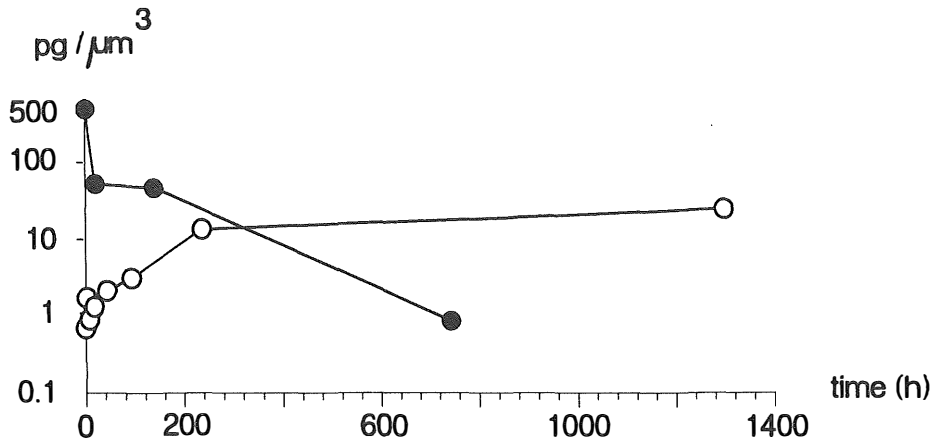

Fig. 3. Protein concentrations per volume in pg/µm³ of *D. vulgaris* (●) and *L. anguillarum* (○) under anaerobic starvation.

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