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Limitations of nalidixic acid dependent viable direct counts in sediment samples

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Abstract

A direct microscopic method based on the response of bacterial cells to inhibition of DNA synthesis by nalidixic acid in the presence of growth-supporting yeast extract and designed to determine the number of viable bacteria, was tested in marine sediments bioturbated by the deposit-feeding polychaete *Arenicola marina*. The number of responsive cells in sediment samples ingested and egested by the polychaete, reflected similar differences and trends as total direct counts or plate counts. Nevertheless, application of that viable direct count technique in marine sediments suffered also from considerable systematic errors.

Introduction

Sediment bacteria are stimulated by deposit feeding polychaetes (HYLLEBERG 1975, PLANTE et al. 1989). On the other hand, most of the total bacterial biomass of ingested sediment is removed by the same macro-invertebrates (GROSS-MANN and REICHARDT, ms. submitted). To determine the abundance of living cells among the total numbers of bacteria in bioturbated sediment structures in which microsites of copiotrophic bacterial growth may occur next to starved populations (e.g. PLANTE et al. 1989), a reliable measure of bacterial viability is needed.

Because of the low level of detection of indirect techniques such as plate counts, the viable direct count (V.D.C.) technique (KOGURE et al. 1979) was taken into consideration. This assay aims primarily at non-exacting copiotrophic bacteria. Its application in "feast and famine" habitats like bioturbated sediments appeared therefore most promising. In seawater, direct viable counts may exceed plate counts by a factor of 1000 and account for 2 - 83 % of the total bacterial populations (KOGURE et al. 1979, PEELE and COLWELL 1981).

Material and methods

Burrows of *Arenicola marina* were dug out and sampled on intertidal flats of the North Frisian Wadden Sea at Westerhever Sand (Eiderstedt peninsula).

Sediment samples (0.1 cm³) were withdrawn using sawed-off 1.0 ml-syringes, suspended in 1 ml of sterile artificial seawater (ASW), subject to ultrasonic treatment (3 x 5 s at 50 W) and diluted up to 10 000-fold in sterile filtered ASW. While preserving part of this suspension with 4 % formaldehyde (final conc.) for reference estimates of AODC (DALEY and HOBBIE 1975, REICHARDT 1988), the non-fixed part of the suspension was incubated for 24 h at 20 °C with 0.02 % (final conc.) of sterile filtered nalidixic acid (NA) and 0.25 % of autoclaved yeast extract. Following membrane filtration onto irgalane black-stained 0.2 µm-Nuclepore filters and preparation of microscopic slides, the elongated red-orange-stained cells were counted as VDC using an epifluorescence microscope (Leitz Dialux 20) in the same way as for AODC (Acridin-Orange-Direct-Counts).

Results and discussion

A sediment sample from the oesophagus region of the polychaete incubated for 24 h, showed no significant difference between total cell densities (Fig. 1). This

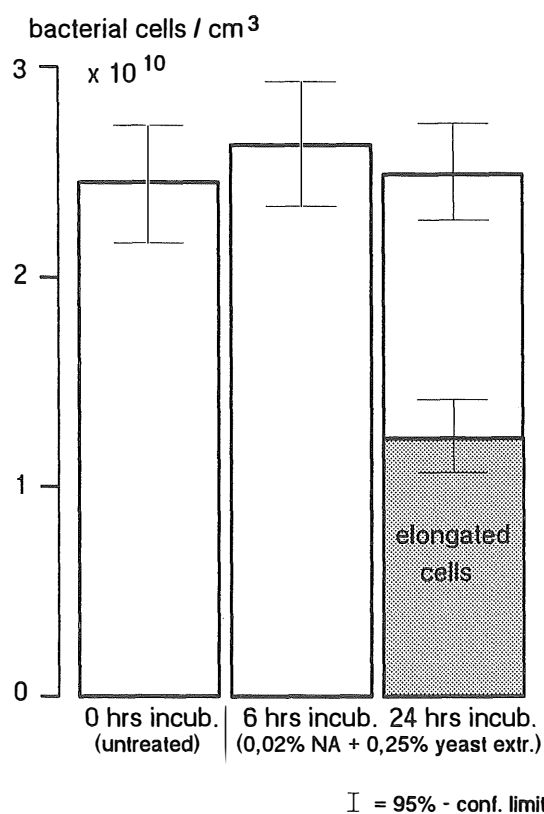


Fig. 1. Total AODC and number of enlarged cells counted after different times of exposure to nalidixic acid and yeast extract. Source: sediment suspension from oesophagus of *A. marina*.

indicated that in this case 0.02 % nalidixic acid (NA) prevented cell divisions effectively. Elongated cells as criterion for viability appeared after 24 h of incubation, however, not yet after six hours as noted for seawater samples in the presence of only 0.002 % of NA (KOGURE et al. 1979, PEELE and COLWELL 1981). Hence, it was necessary to modify the original technique when being applied to marine sediment samples.

Earlier observations had indicated drastic declines of total bacterial numbers after ingestion (GROSSMANN and REICHARDT, ms. submitted). This trend was essentially confirmed by VDC (elongated cells) being significantly ($P=0.01$) lower on the egestive side (hind gut and fecal casts) than on the ingestive side (funnel, food chamber and oesophagus) (Fig. 2). This drop of VDC could be explained by losses of bacterial viability in the digestive tract of the polychaete. Apparently, certain bacterial cells had been able to escape resorption, but had lost their ability to divide. Similar conclusions could also be drawn from plate counts.

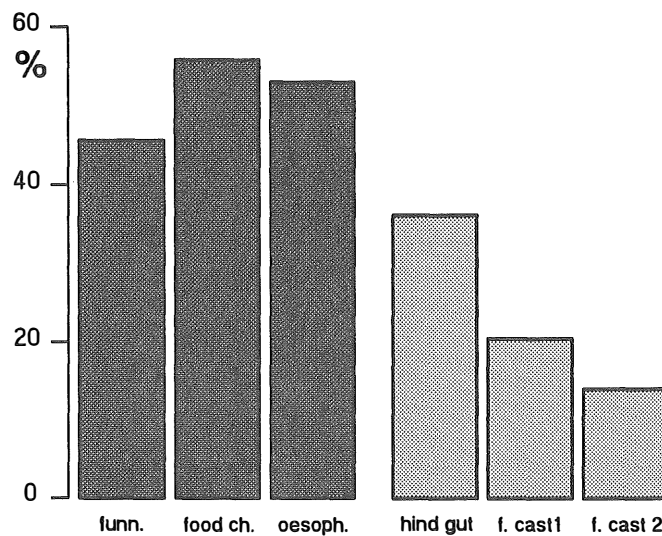


Fig. 2. Viable direct counts (VDC) of enlarged cells after 24 h of incubation of sediment from different sampling sites of *Arenicola marina* burrow systems (mean values of $n = 5$ burrows).

Nevertheless, the VDC's obtained from sediment samples must be interpreted with particular caution due to unpredictable systematic errors. In contrast to previous observations (Fig. 1), complete cessation of cell divisions during 24 h of incubation was not always warranted. Compared with reference determinations of AODC's in parallel subsamples that had been fixed immediately, the total cell density could rise to several hundred percent of the initial value before incubation (Fig. 3). Hence, even 10-fold enhanced concentrations of NA were not sufficient to ensure complete cessation of cell divisions during incubation. This result is not surprising in view of the wide range of concentrations of NA reported to terminate cell division (GOSS and COOK 1975, PEDRINI 1979). Furthermore,

possible adsorption or inactivation of NA in the supernatant of the settled suspensions of the sediment subsample applied has also to be taken into account. Finally, in view of the enormous physiological heterogeneity of microbial communities in sediments, it appears quite plausible that apparently not all bacterial cells had responded to inhibition by NA.

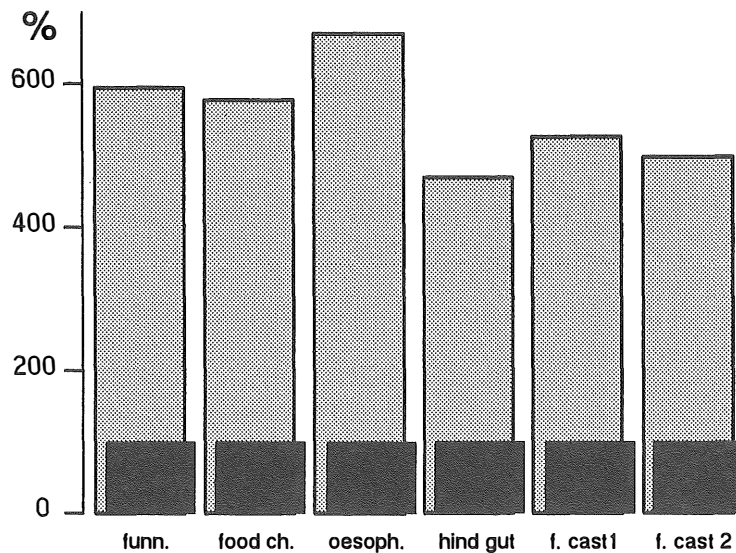


Fig. 3. Viable direct counts of total cells (enlarged and normal) obtained from *Arenicola marina* burrows and plotted as percentage of bacterial densities in untreated reference samples (n = 5).

In conclusion, it can be stated that, although certain inadequacies of this VDC technique have already been pointed out by its former users (KOGURE et al. 1979, PEELE and COLWELL 1981), further systematic errors are likely to be encountered when the method is applied to sediment samples.

References

- DALEY, R.J. and J.E. HOBBIIE, 1975. Direct counts of aquatic bacteria by a modified epifluorescence technique. *Limnol. Oceanogr.* 20, 875-882.
- GOSS, W.A. and T.M. COOK, 1975. Nalidixic acid - mode of action. In: J.W. CORCORAN and F.E. HAHN (eds.), *Antibiotics*. Vol. 3. Springer Verlag, Berlin.
- HYLLEBERG, J., 1975. Selective feeding by *Abarenicola pacifica* with notes on *Abarenicola vagabunda* and a concept of gardening in lug worms. *Ophelia* 14, 113-137.
- KOGURE, K., U. SIMIDU and N. TAGA, 1979. A tentative direct microscopic method for counting living marine bacteria. *Can. J. Microbiol.* 25, 415-420.

- PEDRINI, A.M., 1979. Nalidixic acid. In: F.E. HAHN (ed.), Antibiotics. Vol. 5. Springer Verlag, Berlin, 154-175.
- PEELE, E.R. and R.R. COLWELL, 1981. Application of a direct microscopic method for enumeration of substrate-responsive marine bacteria. Can. J. Microbiol. 27, 1071-1075.
- PLANTE, C.J., P.A. JUMARS and J.A. BAROSS, 1989. Rapid bacterial growth in the hindgut of a marine deposit feeder. Microb. Ecol. 18, 29-44.
- REICHARDT, W., 1988. Impact of bioturbation by *Arenicola marina* on microbiological parameters in intertidal sediments. Mar. Ecol. Prog. Ser. 44, 149-158.