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RELATIONSHIP BETWEEN VIABLE AND DIRECT BACTERIOLOGICAL COUNTS IN SOUTHERN POLAR MARINE WATERS

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TOTAL BACTERIA
HETEROTROPHIC BACTERIA
ANTARCTICA

ABSTRACT – While microscopic direct counts are related to total bacterial biomass, viable bacterial counts are assumed to be representative of only a relatively small part of seawater bacteria. Such an assumption is certainly true in conditions of low nutrient concentrations, but must be considered carefully in more eutrophic situations as showed in this paper. Great similarities between viable and direct bacterial counts were indeed observed from several studies in southern polar zones.

BACTÉRIES TOTALES
BACTÉRIES HÉTÉROTROPHES
ANTARCTIQUE

RÉSUMÉ – Alors que les comptages microscopiques directs sont reliés à la biomasse bactérienne les comptages viables sont considérés comme représentatifs d'une proportion relativement faible des microflores bactériennes marines. Une telle observation est certainement vérifiée dans les milieux oligotrophes mais doit être considérée avec précautions dans des milieux soumis à des conditions plus eutrophiques. Une grande similarité entre comptages bactériens totaux et viables est en effet observée dans plusieurs situations eutrophes rencontrées en milieu polaire austral.

INTRODUCTION

Recent advances on counts technology have led to increase the notion of bacterial biomass necessary to understand the trophic food webs (Horowitz *et al.* 1983). Total bacterial numbers or biomass determinations yield valuable information on standing stock without however reflecting the real metabolic activity (review by Es Van & Meyer-Reil 1982). One of the concerns in microbial ecology is to elucidate the relation between the active viable bacteria and the total bacterial community. It is obvious that the plate count method is insufficient to obtain a realistic picture of active bacteria (Zobell 1946, Jannasch & Jones 1959). One of the first report of the large discrepancy between viable and total direct counts was that of Razumov (1932). The high ratios between these counts are explainable in part by the predominancy of obligate oligotrophs which cannot grow on rich media (Ishida *et al.* 1986). Excellent reviews on the subject are provided by Es Van & Meyer-Reil (1982) and Staley & Konopka (1985). Some approaches using autoradiography (Meyer-

Reil 1978), tetrazolium salts reduction (Zimmermann *et al.* 1978) or nalidixic acid inhibition (Kogure *et al.* 1979) reveal that 10 % of the bacteria counted by microscopic methods are usually alive in the aquatic environment.

This note focuses on a comparison of the ratio between viable counts (V.C.) and direct counts (D.C.) in southern polar marine waters where drastic modifications of the environment (from low nutrient to high nutrient concentrations) induce significant growth of viable heterotrophic bacteria (Delille & Bouvy 1989).

MATERIALS AND METHODS

The bacterioplankton was sampled in Adélie Land (6640'S; 14001'E) and in Kerguelen Archipelago (4923'S; 7012'E). Site description and details of water sampling procedures were previously reported (Delille 1987, Bouvy & Delille 1988). Total bacteria were enumerated by the direct microscopic method according to Hobbie *et*

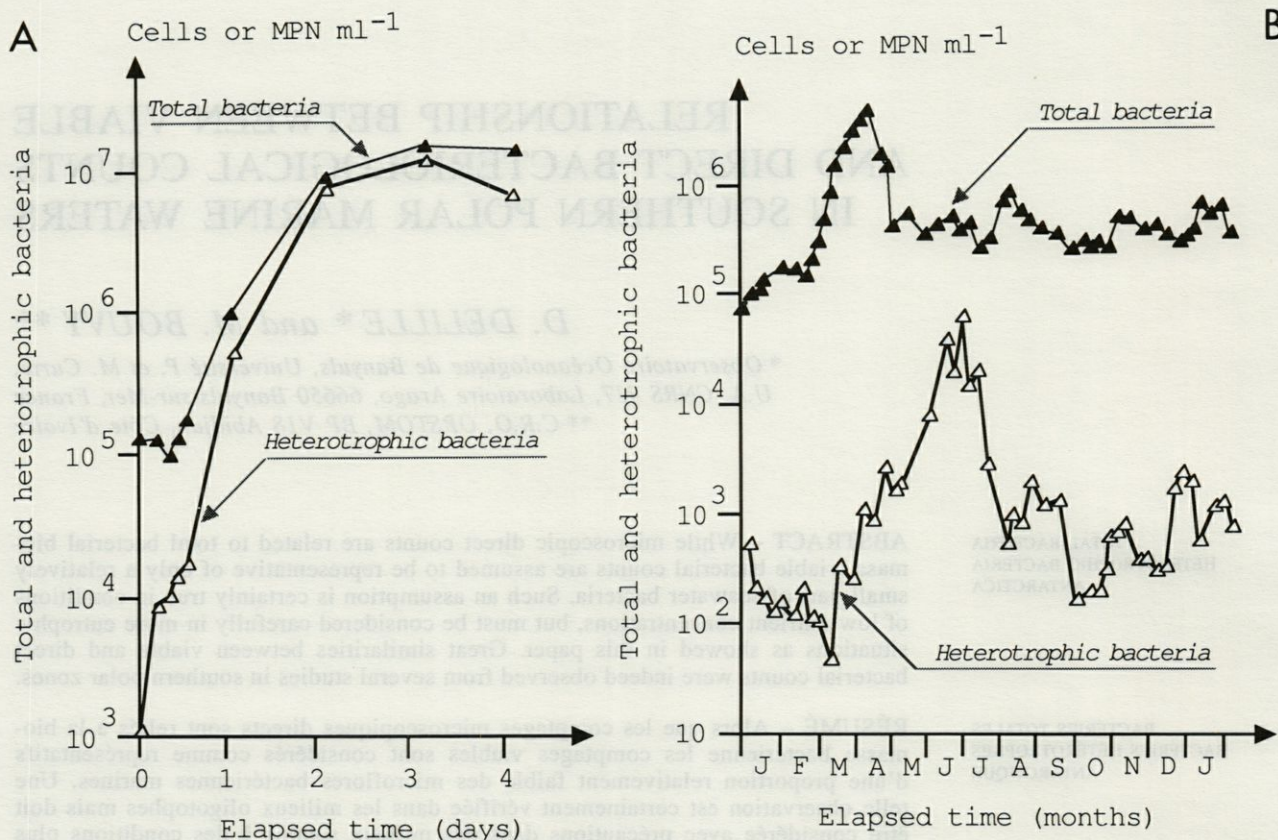


Fig. 1. - A, daily variations in direct and viable bacterial counts in enriched batch experiments conducted with subAntarctic coastal seawater; B, seasonal variations in direct and viable bacterial counts in subAntarctic surface seawater.

al. (1977). Viable heterotrophic bacteria were estimated using the 2216 E medium (Oppenheimer & Zobell 1952) which is considered as the conventional medium in marine microbiology (Ishida *et al.* 1981). Previous studies in southern polar zones showed that the most probable number (MPN) method (6 tubes per dilution) gave bacterial counts one order of magnitude higher than the plate counts (Imbaud 1987); this fact was also reported by Simidu *et al.* (1986) in similar environment. Thus, the application of this technique was preferred for the quantitative determinations of viable heterotrophic bacterial community. All the inoculates were incubated at 18 C for 20 days. In the investigated polar regions, this temperature allowed to reduce the incubation time without significant alteration of the final counts (Delille *et al.* 1988, Delille & Perret 1989).

RESULTS AND DISCUSSION

Our previous short or long term studies showed that the changes in the viable heterotrophic bacterial abundances were always much greater than those of the total bacterial community. The sea-

sonal variation of heterotrophic bacterial numbers generally exceeded three orders of magnitude while total bacterial counts did not vary on average over more than one order of magnitude in the natural environment. From these previous investigations, three examples were chosen to illustrate our point: two natural surveys (Fig. 1 and 2) and one experiment of artificial enrichment (Fig. 1 B). In the absence of specific enrichments such as penguin manuring in the Antarctic area (Delille 1987) or macrophytes decay in the subAntarctic area (Bouvy *et al.* 1986) or before artificial supply during experiments (Fig. 1 A), natural polar conditions are generally considered as nutrient-poor conditions. Overall values of D.C./V.C. ratio range from 100 to 1000.

However, when obvious and significant organic inputs occurred, we always observed lower D.C./V.C. ratios due to sudden increases of heterotrophic viable counts (Delille & Bouvy 1989). SubAntarctic bacterioplankton surveys clearly demonstrated this phenomenon as revealed during the autumnal months (April to July; Fig. 1 B). Natural organic inputs such as penguin manuring in the Antarctic coastal water induced an increase of the viable counts (Fig. 1); this obvious growth of bacteria was significantly correlated with the

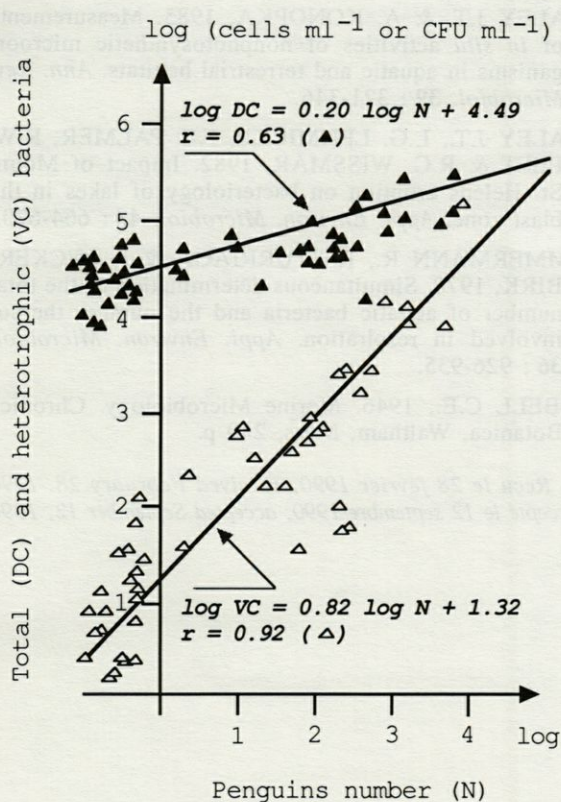


Fig. 2. - Correlation between total bacterial counts (D.C.), viable heterotrophic counts (V.C.) and the penguin number (N) in Antarctic coastal seawater.

penguin numbers (Delille 1987). Organic inputs can be artificially simulated (Fig. 1 A); the changes in bacterial counts were followed in the subAntarctic seawater for 4 days during batch experiments with organic carbon supplement at the concentration of 100 mg.l⁻¹ (Delille & Cahet 1984). Similarities between viable and direct counts appeared already after 36 hours at the *in situ* temperature. The low C.D./V.C. ratios suggest that a fairly large proportion of the bacterial population in southern polar zones would have the ability to grow actively in natural or artificial eutrophic habitats. A similar observation was reported by Staley *et al.* (1982) after the Mount St. Helens eruption; after, the lakes in the blast zone were rapidly transformed from «oligotrophy» to «eutrophy» by the eruption, viable enumerations provided growth of up to 81.5 % of the total bacteria.

Although the changes observed in D.C./V.C. ratio are hardly interpretable, they may indicate realistic modifications of the bacterial communities. Thus, even if direct counts provide the best picture of bacterial biomass, this parameter does not necessarily reflect the metabolic activity of the total bacterial community (Es Van & Meyer-Reil 1982). The results of the viable bacterial counts greatly depend on the counting methods used but they can give complementary information on the

physiological state of the total bacterial community which is not simply revealed by other bacteriological methods.

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REFERENCES

- BOUVY M., M. LeROMANCER & D. DELILLE, 1986. Significance of microheterotrophs in relation to the degradation process of subAntarctic kelp beds (*Macrocystis pyrifera*). *Polar Biol.* 5 : 249-253.
- BOUVY M. & D. DELILLE, 1988. Spatial and temporal variations of subAntarctic and Antarctic bacterioplankton. *Neth. J. Sea Res.* 22 : 139-147.
- DELILLE D., 1987. Spatial distribution of coastal Antarctic seawater bacteria : relationship with avifauna. *Polar Biol.* 8 : 55-60.
- DELILLE D. & M. BOUVY, 1989. Bacterial responses to natural inputs in a marine subAntarctic area. *Hydrobiologia* 182 : 225-238.
- DELILLE D. & G. CAHET, 1984. Croissance de populations hétérotrophes subantarctiques soumises à des enrichissements azotés. Effets des hydrocarbures. (Ed) CNRS Paris, Bact. Mar., Mai 1982, p. 213-219.
- DELILLE D. & E. PERRET, 1989. Influence of temperature on the growth potential of southern polar marine bacteria. *Microb. Ecol.* 18 : 117-123.
- DELILLE D., M. BOUVY & G. CAHET, 1988. Short-term variations of bacterioplankton in Antarctic zone : Terre Adélie area. *Microb. Ecol.* 15 : 293-309.
- ES VAN F.B. & L.A. MEYER-REIL, 1982. Biomass and metabolic activity of heterotrophic marine bacteria. *Adv. Microbiol. Ecol.* 6 : 111-170.
- HOBBIE J.E., R.J. DALEY & S. JASPER, 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33 : 1225-1228.
- HOROWITZ A., M.I. KRICHEVSKY, R.M. ATLAS, 1983. Characteristics and diversity of subarctic marine oligotrophic, stenoheterotrophic, and euryheterotrophic bacterial populations. *Can. J. Microbiol.* 29 : 527-535.
- IMBAUD P., 1987. Dynamique saisonnière d'une communauté bactérioplanctonique subAntarctique (Archipel de Kerguelen). Thèse Univ. Lyon, 139 p.
- ISHIDA Y., H. KADOTA, 1981. Growth patterns and substrate requirements of naturally occurring obligate oligotrophs. *Microb. Ecol.* 7 : 123-130.
- ISHIDA Y., M. EGUCHI & H. KADOTA, 1986. Existence of obligately oligotrophic bacteria as a dominant population in the South China Sea and the West Pacific Ocean. *Mar. Ecol. Prog. Ser.* 30 : 197-203.
- JANNASCH H.W. & G.E. JONES, 1959. Bacterial populations in seawater as determined by different methods of enumeration. *Limnol. Oceanogr.* 4 : 128-129.

KOGURE K., U. SIMIDU, N. TAGA, 1979. A tentative direct microscopic method for counting living marine bacteria. *Can. J. Microbiol.* **25** : 415-420.

MEYER-REIL L.A., 1978. Autoradiography and epifluorescence microscopy combined for the determination of number and spectrum of actively metabolizing bacteria in natural waters. *Appl. Environ. Microbiol.* **36** : 506-512.

OPPENHEIMER C.H. & C.E. ZOBELL, 1952. The growth and viability of sixty three species of marine bacteria as influenced by hydrostatic pressure. *J. Mar. Res.* **11** : 10-18.

RAZUMOV A.S., 1932. Interrelation between bacteria and plankton in connection with some problems of water hygien. *Mikrobiol.* **1** : 131-146.

SIMIDU U., K. KOGURE, K. FUKAMI & C. IMADA, 1986. Heterotrophic bacterial flora of the Antarctic ocean. *Mem. Natl Inst. Polar Res.* **40** : 405-412.

STALEY J.T. & A. KONOPKA, 1985. Measurements of *in situ* activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Ann. Rev. Microbiol.* **39** : 321-346.

STALEY J.T., L.G. LEHMICKE, F.E. PALMER, R.W. PEET & R.C. WISSMAR, 1982. Impact of Mount St. Helens eruption on bacteriology of lakes in the blast zone. *Appl. Environ. Microbiol.* **43** : 664-670.

ZIMMERMANN R., R. ITURRIAGA & J. BECKER-BIRK, 1978. Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. *Appl. Environ. Microbiol.* **36** : 926-935.

ZOBELL C.E., 1946. Marine Microbiology. Chronica Botanica, Waltham, Mass, 240 p.

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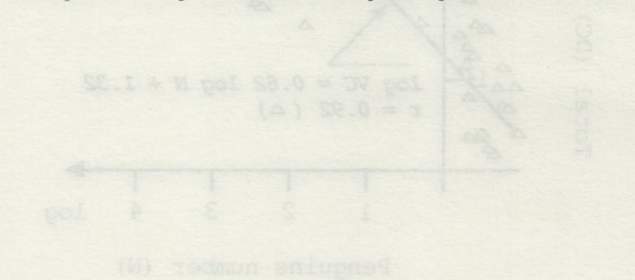


Fig. 2 - Correlation between total bacterial counts (log N) and viable heterotrophic counts (log V.C.) in Antarctic coastal seawater.

DELILLE D. 1987. Spatial distribution of coastal Antarctic seawater bacteria: relationship with salinity. *Mar. Biol.* **8** : 25-30.

DELILLE D. & M. BOUVY, 1989. Bacterial responses to natural inputs in a marine subantarctic area. *Hydrobiologia* **185** : 223-238.

DELILLE D. & G. CAHET, 1984. Coexistence de populations bactériennes saprophytiques soumise à des enrichissements azotés. Effets des hydrostatiques. (Ed) CNRS Paris, Rep. Mar. Mar. 1985, p. 213-218.

DELILLE D. & H. PÉRETT, 1989. Influence of temperature on the growth potential of southern polar marine bacteria. *Microp. Écol.* **18** : 117-123.

DELILLE D., M. BOUVY & G. CAHET, 1988. Short-term variations of bacterioplankton in Antarctic zone: Tercé Adélie area. *Microp. Écol.* **15** : 293-303.

ES VAN DER, J. A. MEYER-REIL, 1982. Biomass and metabolic activity of heterotrophic marine bacteria. *Mar. Microbiol. Écol.* **6** : 111-170.

HOBBS J.E., R.J. DALRY & S. JASPER, 1977. The Neohogon filter for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* **35** : 1223-1228.

HOROWITZ A., M.I. KRICHEVSKY, K.M. ALAN, 1983. Characteristics and diversity of subantarctic oligotrophic, mesoheterotrophic, and euryhaline trophic bacterial populations. *Can. J. Microbiol.* **29** : 527-532.

IMBARD P., 1987. Dynamique saisonnière d'une communauté bactérienne subantarctique. *Arch. Hydrobiol. (Suppl.)* **139** p.

ISHIDA Y., H. KADOTA, 1981. Growth patterns and substrate requirements of naturally occurring oligotrophic bacteria. *Microp. Écol.* **7** : 123-130.

ISHIDA Y., M. BOUCHI & H. KADOTA, 1986. Ecological characteristics of oligotrophic bacteria as a dominant population in the South China Sea and the West Pacific Ocean. *Mar. Biol.* **90** : 187-201.

JANNASCH H.W. & G.E. JONES, 1959. Bacterial populations in seawater as determined by different methods of enumeration. *Limnol. Oceanogr.* **4** : 128-139.

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