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DISTRIBUTION/ GROWTH TEMPERATURES, AND GRAM REACTIONS OF PSYCHROPHILIC SEDIMENT BACTERIA FROM TROPICAL AND SUBTROPICAL REGIONS OFF WEST-AFRICA

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ABSTRACT - In depths greater than 1 000 m viable counts of sediment bacteria from the continental slope off West-Africa were significantly higher after incubation of agar plates at 2°C than at 20°C. At a depth of 1 500 m in 17°N, for example, 438 000 cells/ml of wet sediment were found after incubation at 2°C, but at 20°C the counts were only 54 000 cells/ml. In the deep-sea of the eastern tropical Atlantic bacterial counts were low, between 70 and 14 000 cells/ml of wet sediment, and about the same for both incubation temperatures. However, different types of bacteria were isolated, depending upon the incubation temperature applied. Most of the bacteria isolated from 20°C agar plates were Gram-positive and mesophilic. In contrast, the majority of the isolates from 2°C plates were Gram-negative and extremely psychrophilic. Their optimal growth temperature was about 4°C and the maximal growth temperature ranged from 4 to 12°C. The results demonstrate that psychrophilic bacteria occur abundantly in marine sediments, not only in the polar regions, but also elsewhere in the sea.

Key words : marine bacteria, psychrophilic bacteria, deep-sea, sediment,tropical Atlantic.

RÉSUMÉ - A des profondeurs supérieures à 1 000 m, le nombre des bactéries viables du sédiment de la pente continentale, au large de l'Afrique de l'Ouest, est significativement plus élevé après incubation sur boîtes d'agar, à 2°C qu'à 20°C. Par exemple, à la profondeur de 1 500 m et à la latitude de 17°N, 438 000 cellules/ml de sédiment humide sont dénombrées après incubation à 2°C. A 20°C, ce nombre n'est que de 54 000 cellules/ml. Dans les eaux abyssales de l'Atlantique tropical Est, le nombre de bactéries est faible, entre 70 et 14 000 cellules/ml de sédiment humide, et est resté à peu près du même ordre de grandeur pour les deux températures d'incubation. Toutefois, différents types de bactéries sont isolées en fonction de la température d'incubation. La plupart des bactéries isolées sur les boîtes incubées à 20°C sont Gram positif et mésophiles. Au contraire, la majorité des isolats effectués sur des boîtes incubées à 2°C sont Gram négatif et fortement psychrophiles. Leur température optimale de croissance est de 4°C et leur température maximale entre 4 et 12°C. Ces résultats montrent que les bactéries psychrophiles sont abondamment présentes dans les sédiments marins, non seulement dans les régions polaires, mais également dans d'autres environnements marins.

Mots clés : bactéries marines, bactéries psychrophiles, abysses, sédiment, Atlantique tropical.

INTRODUCTION

Deep-sea temperatures are generally lower than 3°C. However, studies about the size and structure of culturable psychrophilic bacterial communities from the deep-sea have only been reported from polar and subpolar regions (Norkrans and Stehn, 1978 ; Kaneko *et al.*,1979 ; Tanner and Herbert, 1982) but not from tropical and subtropical areas. Psychrophilic and psychrotrophic bacteria according to Morita (1975) have been isolated from various materials and locations of the sea (Wirsen and Jannasch, 1975 ; Baross and

Morita, 1978 ; Tabor *et al.*, 1982). Extreme psychrophiles which failed to grow at temperatures in excess of 10°C are known from the deep Norwegian Sea (Norkrans and Stehn, 1978) and from Antarctic waters (Baross and Morita, 1978). Yayanos and Dietz (1982) reported the inactivation of a barophilic deep-sea bacterium from the central North Pacific ocean after exposures to temperatures between 10 and 32°C. Thus, the incubation temperature of 20°C, originally thought to allow the isolation of both mesophilic and psychrophilic bacteria (Harder and Veldkamp, 1968 ; Bensoussan et al., 1979), inhibits the growth of autochthonous deep-sea bacteria having lower maximum growth temperatures. Besides 20°C, we therefore applied a second incubation temperature of 2°C for quantitative estimations and isolations of psychrophilic and mesophilic sediment bacteria in the upwelling area off West-Africa (Rüger, 1982) and in the deep-sea of the eastern equatorial Atlantic.

MATERIALS AND METHODS

Viable counts of psychrophilic and mesophilic sediment bacteria have been determined in the upwelling area off NW-Africa and in the deep-sea of the eastern equatorial Atlantic by using the spread plate method. The nutrient agar consisted of peptone, 1.5 g, yeast extract, 0.3 g, $FePO_4.4H_2O$, 0.01 g, agar, 15.0 g, seawater, 750 ml, and distilled water, 250 ml ; pH 7.6. Serial dilutions of sediment from the topmost layer (0 - 2 cm) were prepared with 75 % seawater. The agar plates and seawater solutions were chilled to 4°C before use. Immediately after recovery of the box corer or van Veen grab, the spread plate cultures were prepared. A cold tray was used to ensure that the sample and agar plate temperatures did not exceed 4°C during the inoculation procedures. Four plates per dilution were incubated for 12 and 4 weeks at 2°C and 20°C, respectively. The positions of sampling stations at the 60 th and 65 th cruises of RV "Meteor" in 1982 and 1983 are given in Fig. 1-4. Bacterial strains were isolated from both the 2°C and 20°C plates. The Gram reactions of the strains were determined with the nonstaining KOH method described by Buck (1982). The optimal and maximal growth temperatures of the 2°C isolates were determined by incubation at 4, 12, 18 and 24°C. Growth was recorded after 3,7 and 14 days of incubation in a nutrient broth, having the same constituents as the nutrient agar.

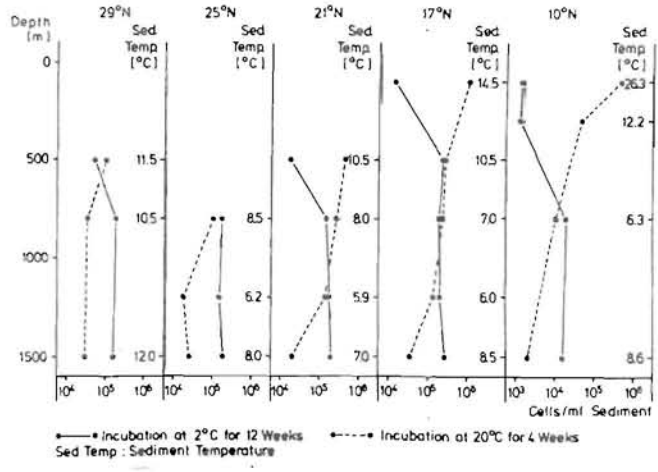

Figure 1 : Viable counts of psychrophilic and mesophilic bacteria in sediment samples of the West-Africa continental slope. Incubation at 2°C for twelve weeks and at 20°C for four weeks on 1/3 Zobell-Agar.

RESULTS

In depth between 1 000 and 1 500 m at the continental slope, the viable counts at 2°C were significantly higher than the counts at 20°C (Fig. 1). At the 1 500 m station in 17°N, for example, we found 54 000 cells/ml of wet sediment after incubation at 20°C, but the counts were 438 000 cells/ml at 2°C.

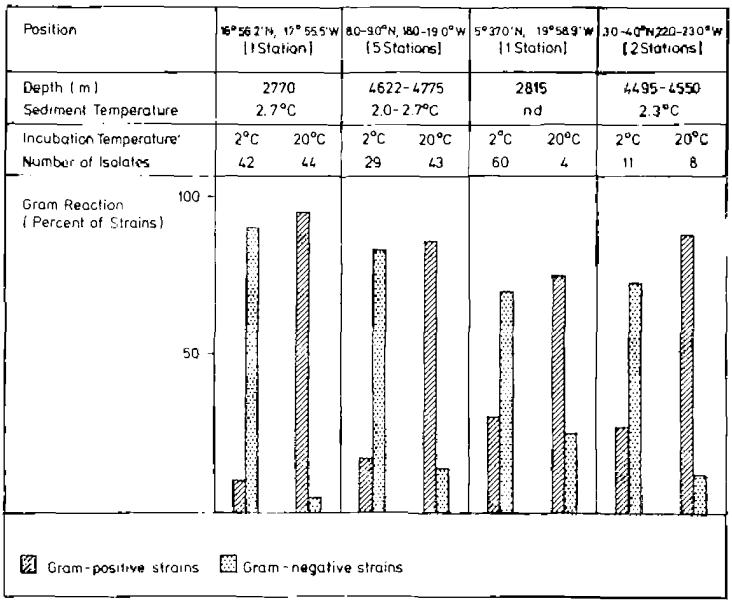

Figure 2 : Gram-reactions of deep-sea bacteria, isolated fro 2°C and from 20°C plates.

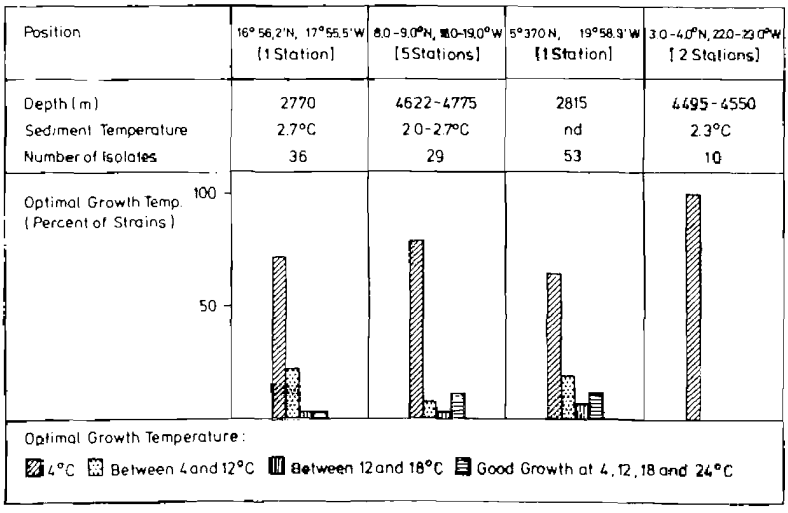

Figure 3 : Optimal growth temperatures of deep-sea bacteria, isolated from 2°C plates.

In deep-sea samples from depths of between 2 770 and 4 775 m, viable counts were low, 70 to 14 000 cells/ml of wet sediment, and about the same for both incubation temperatures. However, different types of bacteria were isolated dependent on the incubation temperature applied. The majority of the isolates from the 2°C plates were Gram-negative, but most of the 20°C isolates belonged to the Gram-positive type (Fig. 2).
The majority of the deep-sea isolates from the 2°C plates were extremely psychrophilic. Their optimal growth temperatures were about 4°C (Fig. 3) and their maximal growth temperatures ranged from 4 to 12°C (Fig. 4).

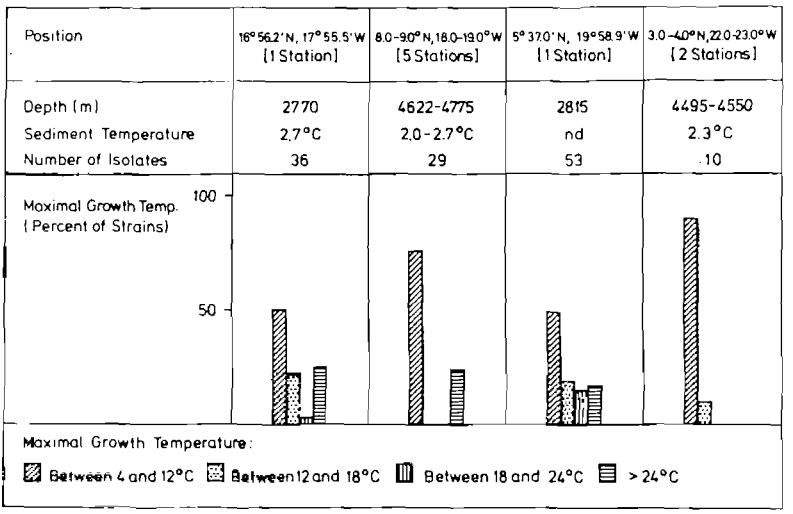

Figure 4 : Maximal growth temperatures of deep-sea bacteria, isolated from 2°C plates.

DISCUSSION

In the past, incubation temperatures of about 20°C have been used to determine numbers of culturable bacteria in the sea. The majority of deep-sea sediment bacteria, isolated from 20°C plate cultures, belonged to the Gram-positive type (Rüger, 1973 ; Bensoussan *et al.*,1979, 1981). Corresponding results were obtained from surface sediment samples of the eastern tropical Atlantic (Fig.2). Most of the Gram-positive isolates are mesophilic, did not grow at 4°C within six weeks, and were able to grow in freshwater media (unpublished data). Since these organisms are not adapted to low temperatures, they are probably not indigenous to the deep-sea environment.
On the other hand, most of the deep-sea isolates from 2°C plates are Gram-negative and psychrophilic. The maximal growth temperatures for 75 strains from the topmost sediment layers were between 4 and 12°C, for 19 strains between 12 and 18°C, and for 9 strains between 18 and 24°C. Only 25 strains were able to grow at 24°C. The results demonstrate that it is essential to use in situ temperatures for the isolation of native deep-sea bacteria, as already discussed by Yayanos and Dietz (1982), and that psychrophiles occur abundantly in marine sediments, not only in the polar regions, but also elsewhere in the sea.

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