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Benthic studies of the northwest African upwelling region: psychrophilic and psychrotrophic bacterial communities from areas with different upwelling intensities*

Hans-Jürgen Rüger

Alfred-Wegener-Institut für Polar- und Meeresforschung, D-2850 Bremerhaven, Federal Republic of Germany

ABSTRACT: Distinct bacterial communities were found in the sediments of the northwest African upwelling area at 17 and 21°N compared to an area with low upwelling intensities at 29 and 35°N. The bacterial community of the central upwelling area at 17/21°N consisted of approximately equal proportions of Gram-negative strains belonging mainly to the genera *Alteromonas* and *Vibrio*; most of them were psychrophiles. This could be due to the upwelling of cold water masses in this region, where sediment temperatures were on average 2°C lower than in the northern part of this upwelling area. Here, Gram-positive psychrotrophic *Bacillus* strains predominated, tolerating growth temperatures higher than 24°C. A high proportion of these *Bacillus* strains fermented various carbohydrates and sugar alcohols and utilized citrate as sole source of carbon and energy. In contrast, the abilities to utilize some glycosides, starch, chitin, fats and proteins, to degrade various N-compounds like DNA or arginine, and to reduce nitrates to nitrites were more pronounced in strains from the central upwelling area.

INTRODUCTION

Upwelling causes an enhanced primary productivity rate in water masses of the northwest African continental margin. The centre of this upwelling is found in the Cape Blanc area at 21°N throughout the year. Seasonal upwelling occurs south of Cape Blanc to Sierra Leone in winter and spring and to the north up to Morocco in summer (Fütterer 1983).

Studies on benthic activity in upwelling regions are extremely rare, although it is known that primary production of the overlying waters strongly influences benthos standing stock and productivity rate (Thiel 1981). As can be expected, a high biomass has been found in the Cape Blanc area and decreasing values to the north and south of it (Thiel 1982, Pfannkuche et al. 1983, Tan & Rüger 1989).

Bacteriological investigations of sediments from the northwest African upwelling region are also rare in comparison to explorations in the water column. Cell numbers, nutritional properties and taxonomy of planktonic bacteria of the same region have been reported by Meyer-Reil & Rheinheimer (1973), Bölter & Meyer-Reil (1974), Tejero (1978), Tejero et al. (1978) and Zimmermann et al. (1980). Quantitative studies have shown a correlation between decreasing cell numbers of benthic bacteria and increasing water depths (Meyer-Reil & Rheinheimer 1973, Rüger 1975). The distribution and catabolic potentials of different types of sediment bacteria from deep-sea samples of the Cape Blanc area have been investigated by Bensoussan (1979) and Bensoussan et al. (1979). Structures and catabolic potentials of mesophilic bacterial communities at 21°N have been reported (Rüger 1985). We found that *Agrobacterium*-like bacteria predominated in sediments from water depths of 85 to 1023 m. In depths greater than 1300 m, however, the populations consisted mostly of carbohydrate-fermenting *Bacillus* species.

The low deep-sea temperatures, upwelling of cold water masses and existence of psychrophilic bacteria with maximum growth temperatures below 15°C (Baross & Morita 1978, Norkrans & Stehn 1979, Yay-

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anos & Dietz 1982) stimulated us to apply an additional incubation temperature of 2°C besides 20°C during the 60th cruise of RV 'Meteor' in 1982 in the northwest African upwelling region. In contrast to 20°C, this low incubation temperature yielded increasing numbers of culturable sediment bacteria with increasing water depths (Rüger 1982) and led to the isolation of psychrophilic and psychrotrophic bacteria. Physiology, catabolic potentials and taxonomy of these low-temperature-adapted bacterial communities from the Cape Blanc and adjacent areas are described here and compared with mesophilic bacterial strains isolated during a previous cruise of RV 'Meteor' in 1975.

MATERIALS AND METHODS

Sampling methods and station locations. During the cruise SUBTROPEX in January and February 1982 with RV 'Meteor', sediment samples were taken by means of a box-grab sampler from different water depths at 35, 29, 21 and 17°N. Station locations, numbers of grab-samplers and methods of subsampling were presented previously (Tan & Rüger 1989).

Isolation of strains. Viable count determinations at 2 and 20°C were carried out with the spread plate method on seawater agar consisting of 1.5 g peptone, 0.3 g yeast extract, 0.01 g $FePO_4 \cdot 4H_2O$, 15.0 g DIFCO Bacto-agar, 750 ml seawater and 250 ml distilled water. The pH was adjusted to 7.6. Serial dilutions of the samples were prepared in 75% seawater. The agar plates and seawater solutions were chilled to 4°C before inoculation. Immediately after sediment subsampling the spread plate cultures were prepared. A cold tray was used to ensure that the sample and plate temperatures did not exceed 4°C during the inoculation procedure. Four parallel plates were prepared for each incubation temperature and each dilution. The same 2°C plates for enumerating culturable bacteria from the top 2 cm sediment layer were used for isolations of strains at random.

After heating subsamples of the serial dilutions to 80°C for 10 min to inactivate vegetative cells, additional spread plates on spore germination medium II (Rüger 1975) were prepared for enumeration of bacterial spores.

Physiological and biochemical tests. The common identification tests were performed in seawater media as described by Weyland et al. (1970). A modification of the Minitek system was applied for the determination of carbohydrate metabolism (Rüger 1981). Table 1 explaines the abbreviations used in Fig. 3. Tests with psychrotrophic and psychrophilic strains were performed at 12°C; extreme psychrophiles were tested at 4°C. Temperature relationships were determined at 1, 6, 12, 18, 24, 30 and 37°C in seawater broth containing the same concentrations of peptone, yeast extract and $FePO_4 \cdot 4H_2O$ as the seawater agar. Growth was measured at 650 nm with a Gilford spectrophotometer model 250 after 2, 6, 10, 14 and 28 d of incubation.

Table 1. Abbreviations used in Fig. 3

Monosaccharides		
DEX	:	Glucose
LE		Fructose
AR	:	Arabinose
GA	:	Galactose
X	:	Xylose
MA	:	Mannose
R	:	Rhamnose
Di- and Trisaccharides		
TR	·	Trehalose
CE	·	Cellobiose
M	·	Maltose
SU	:	Sucrose
RA	:	Raffinose
ME	:	Melibiose
L		Lactose
Alcohols, Citrate		
G	:	Glycerol
MN	:	Mannitol
CIT	·	Citrate
I	:	Inositol
SO	:	Sorbitol
AD	:	Adonitol
DU	:	Dulcitol
Glycosides		
ES	:	Esculin
ONPG	·	*o*-nitrophenyl-β-D-galactoside
SA	·	Salicin
Polysaccharides		
ST		Starch
CHIT		Chitin
ALG	:	Alginate
Proteins, Lipase		
GEL	:	Gelatin
LIP	:	Lipase (Tributyrin)
CAS	:	Casein
N-compounds		
DNA	·	DNase
ARG	:	Arginine dihydrolase
UR	:	Urease
LYS		Lysine decarboxylase
ORN		Ornithine decarboxylase

The following terms are used to characterize growth temperatures responses of the strains:

Extremely psychrophilic: Optimum growth temperature between 1 and 6°C, maximum growth temperature below 12°C (in some strains, the optimum temperature was between 6 and 12°C, then the maximum temperature for growth was betwen 12 and 18°C).

Psychrophilic: Growth at 1°C, optimum growth

temperature between 6 and 18°, maximum growth temperature between 18 and 24 °C.

Psychrotrophic: Growth at 1 °C, optimum growth temperature between 12 and 18 °C (sometimes higher), maximum growth temperature higher than 24 °C.

Mesophilic: No growth at 5 °C, optimum growth temperature between 18 and 30 °C, maximum growth temperature higher than 24 °C.

RESULTS

Community structures

The agar plates used for enumeration and isolation of benthic bacteria from areas of different upwelling intensities were incubated at 2 °C. This incubation temperature led to the isolation of bacteria adapted to low temperatures. Grouping the strains according to increasing water depths revealed decreasing numbers of psychrotrophic and increasing numbers of psychrophilic bacteria (Fig. 1a). In general, Gram-positive and psychrotrophic bacterial strains predominated at 35 and 29° N. The bacterial communities in areas with high upwelling intensities at 21 and 17° N consisted of Gram-negative strains with mainly psychrophilic growth characteristics (Fig. 1b).

Most of the Gram-positive strains from 35/29° N. belonged to the genus *Bacillus* and only a few strains were assigned to the genus *Micrococcus* or the coryneform group (Fig. 2). The results presented in Table 2 indicate that the greatest part of the *Bacillus* isolates originated from vegetative cells, because only small numbers of resting spores could be demonstrated in the sediment samples. The Gram-negative isolates belonged to the genera *Acinetobacter* (2 %), *Aeromonas* (2 %), *Agrobacterium* (2 %), *Alteromonas* (7 %), *Pseudomonas* (2 %) and *Vibrio* (4 %). Because of their small percentages in all 4 transects, the genera *Acinetobacter, Aeromonas, Agrobacterium* and *Pseudomonas* were – regardless of their taxonomic relatedness-combined in one column in Fig. 2.

The bacterial communities at 21/17° N were Gram-negative and consisted of nearly equal proportions of the genera *Alteromonas* and *Vibrio*; a minority belonged to *Acinetobacter* (2 %), *Agrobacterium* (3 %) and *Pseudomonas* (2 %). The genus *Alteromonas* was differentiated from *Pseudomonas* by seawater base requirement for growth, DNase activity, H_2S-production from cysteine, susceptibility to 10 µg chloramphenicol and 50 µg furazolidone (Oxoid sensitivity disks), and absence of nitrate reduction to gas and of a constitutive arginine dihydrolase system (Gray & Stewart 1980, Baumann et al. 1984).

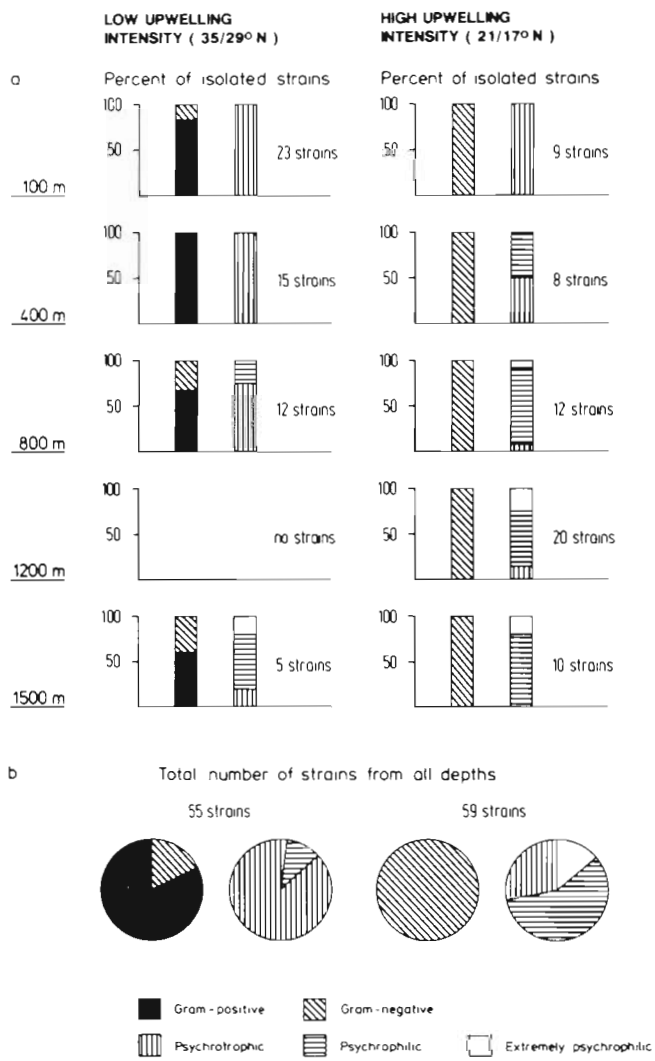

Fig. 1. Gram-reactions and growth temperatures of benthic bacteria from areas of low upwelling intensities (35/29° N) and high upwelling intensities (21/17° N). (a) Gram-reactions and growth temperatures in relation to water depth. (b) Same characteristics for the total number of strains from all depths

Catabolic potentials

A high proportion of isolates from the area with low upwelling intensity were able to ferment various carbohydrates and sugar alcohols and to utilize citrate as sole source of carbon and energy. In the central upwelling region, however, strains possessing these traits were not abundant. Higher metabolic activities, as expected in upwelling regimes, are represented here by higher proportions of strains able to utilize some glycosides, starch, chitin, fats and proteins, to degrade N-compounds like DNA or arginine and to reduce nitrates to nitrites (Fig. 3).

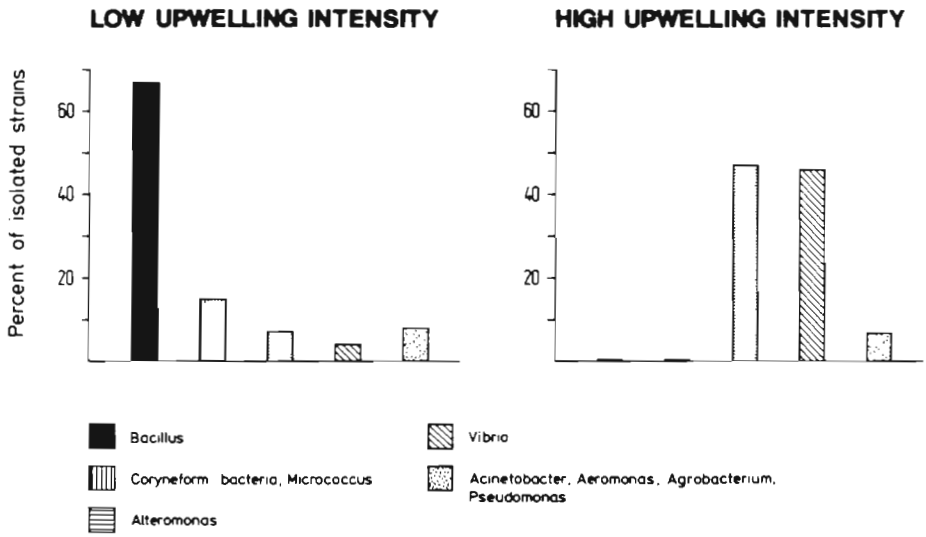

Fig. 2. Taxonomic positions of benthic bacteria from regions with different upwelling intensities

DISCUSSION

No method of investigation is known to obtain a complete insight into the real world of microbial communities in nature (Kölbel-Boelke et al. 1988). Direct microscopic observations for enumeration and determination of morphologies, or following the fate of nutrients and other chemical compounds in the habitat, do not yield enough information about the organisms involved in the ecological processes. In consequence, isolating the bacteria from their environment and further characterizing them in vitro is still an important method. However, it must be born in mind that all isolation procedures are necessarily selective in quality and quantity and that a great proportion of marine bacteria is not culturable at all (Roszak & Colwell 1987). They depend on numerous factors like sample processing, media compositions, temperature and other physical conditions.

The large number of samples needed for an ecological survey results in hundreds of strains to be characterized. Bianchi & Bianchi (1982) calculated that examining 20 to 30 strains is sufficient to obtain a diversity of bacterial community and that the numbers of tests can be reduced to about 30 without any influence on bacterial diversity. The number of strains which could be isolated during the expedition SUBTROPEX '82 was lower than 20 to 30 per sediment sample. The results could therefore not be presented separately for each station or each transect. Therefore, the results of 55 strains from the 2 northern transects were combined and compared to the whole set of

Table 2. Spores and vegetative cells (colony-forming units per ml of wet sediment, incubation at 2 °C) and percentages of *Bacillus* strains from the total number of isolates presented in Fig. 1. Sed.-temp · sediment temperature; nd: not determined

Transect	Depth (m)	Sed.-temp.	Vegetative cells	Spores (% of vegetative cells)	*Bacillus* (% of isolates)
35 °N	138	16.4	123.000	nd	52
	423	12.6	93.000	20	93
	800	10.6	179 000	9	100
29 °N	800	10.5	242.000	1	20
	1491	12.0	209.000	2	60
21 °N	495	10.5	26.000	0.1	0
	780	8.0	142.000	0.1	0
	1198	5.9	241.000	0.4	0
	1471	7.0	307.000	nd	0
17 °N	84	14.5	16.000	0.1	0
	418	10.5	379.000	<0.1	0
	827	7.0	350.000	<0.1	0
	1205	6.0	356.000	0.1	0
	1520	8.5	438 000	<0.1	0

results of the 59 isolates from the central upwelling area. By this procedure, it was possible to demonstrate significant differences between the bacterial communities of the 2 geographical regions (Figs. 1b, 2 and 3). Combining the results from all water depths in these figures was justified by their great similarity in all depths. An example is shown in Table 3 for 2 depth ranges in the central upwelling area. Exceptions were the growth temperature responses of the strains.

Temperature is one of the most important parameters to be considered in marine microbiology. The incubation temperature of 20 °C, still used for bacterial studies of the deep-sea (e.g. Namsaraev 1985), and originally thought to allow the growth of both psychrophilic and the mesophilic bacteria (Harder & Veldkamp 1968, Bensoussan et al. 1979), inhibits the growth of autochthonous bacteria having lower maximum growth temperatures. For example, most of the bacteria from deep-sea samples of the eastern tropical Atlantic were Gram-positive mesophiles when isolated from agar plates incubated at 20 °C. In contrast, the majority of strains from agar plates incubated at ambient temperature of 2 °C were Gram-negative and extremely psychrophilic (Rüger 1986).

The upwelling of cold water masses is reflected by the sediment temperatures in our area of investigation. They decreased with increasing depths and were on average 2 °C lower in the Cape Blanc area than in the 2 northern transects (Table 2). As could be expected, we found higher proportions of psychrophilic and extremely psychrophilic bacteria in the central upwelling area than at 35/29° N; the distribution of psychrophilic bacteria was also dependent on water depth (Fig. 1a, b). The temperature relationships of the strains were tested at 6 °C intervals. Therefore, the maximum growth temperatures between 18 and 24 °C, used here to define psychrophiles, do not completely meet the definition proposed by Morita, who restricted the temperature growth range of psychrophiles from 0 °C, or less, to 20 °C, or less (Baross & Morita 1978). The term 'extremely psychrophilic' was used here to characterize those of our bacteria having even lower maximum growth temperatures than the greatest part of the isolates.

Boje & Tomczak (1978) noted that the high productivity in upwelling regimes seems to be accompanied by a high dominance of a few species (= low diversity). In agreement, we found that the bacterial community in the Cape Blanc area is composed mainly of *Alteromonas* and *Vibrio*. On the other hand, with the predominance of *Bacillus* species, low bacterial diversity was also found at 35/29° N, indicating rather

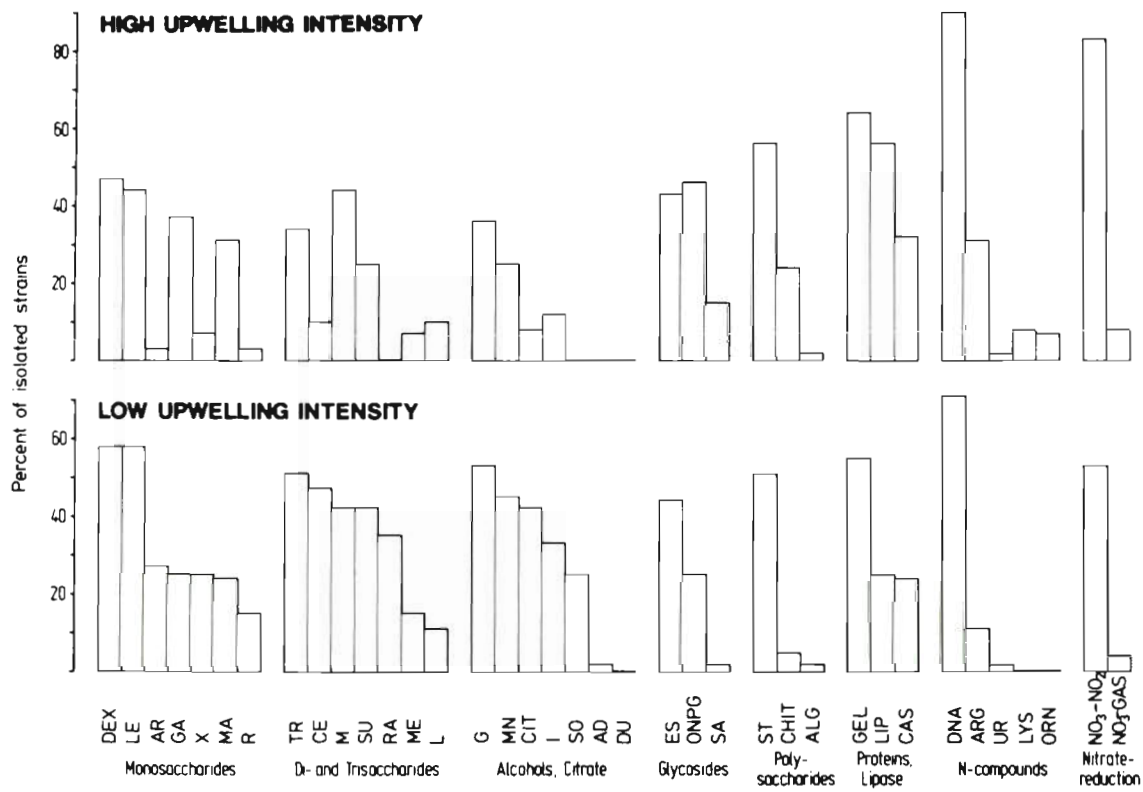

Fig. 3. Catabolic potentials of benthic bacteria from regions with different upwelling intensities (percent of isolates with positive test results)

uniform environmental conditions in regions with low upwelling intensities, too.

The isolates were identified according to Bergey's manual of systematic bacteriology (1984, 1986) and to numerous original publications about the taxonomy of *Alteromonas, Bacillus* and *Vibrio*. The taxonomic characteristics of most of the strains were not in accordance with the descriptions given for any known species. The strains could therefore be identified only to genus level and represent new taxa within the respective genera. Descriptions of these taxa will be published elsewhere.

Species of the genus *Bacillus* were originally soil bacteria and are characterized by spore formation in adverse conditions. However, aerobic sporeformers are also common inhabitants of marine sediments (Rüger 1973, Brisou et al. 1978, Bensoussan et al. 1979, Bonde 1981). *Bacillus marinus* (ex *Bacillus globisporus* subsp. *marinus* Rüger & Richter, 1979) is an obligate marine species (Rüger & Hentzschel 1980, Rüger 1983). Some other *Bacillus* strains from deep-sea sediments required seawater base for growth and grew well at temperatures between 1 and 20°C. They utilized numerous organic substrates at 4°C but not at 20°C and proved to be highly adapted to deep-sea conditions (Rüger 1988).

Bacterial spores are less abundant in marine sediments than vegetative cells (Table 2 and Rüger 1975). It is concluded that the greatest part of our isolates from 35/29° N have evolved from vegetative cells. All the

Table 3. Comparison of bacteria from the Cape Blanc area at 21 °N isolated from spread plates incubated at 15 °C in 1975 and 2 °C in 1982. Strs: strains

Trait	Percent of isolated strains			
	Incubation at 15 °C		Incubation at 2 °C	
	85–1023 m (83 strs)	1318–1745 m (54 strs)	100–800 m (29 strs)	1200–1500 m (30 strs)
Taxonomy				
*Agrobacterium**	87	17	0	7
Bacillus	2	74	0	0
Alteromonas	0	0	59	36
Vibrio	7	0	41	50
Various genera	4	9	0	7
Physiology				
Extremely psychrophilic	0	0	4	23
Psychrophilic	0	0	48	67
Psychrotrophic	31	20	48	10
Mesophilic	69	80	0	0
Growth in freshwater media	31	67	3	13
Acid from				
Glucose	29	70	41	53
Arabinose	1	55	0	7
Fructose	8	69	38	50
Glycerol	13	68	28	43
Lactose	7	33	10	10
Maltose	16	77	41	47
Mannitol	8	60	21	30
Sucrose	19	83	28	23
Xylose	5	55	7	7
Utilization of				
Urea	5	39	0	3
Gelatin	6	26	76	53
Casein	7	26	34	30
Arginine	22	11	31	30
Starch	8	35	52	60
Tributyrin (Lipase)	41	52	62	50
Citrate	12	22	10	7
Chitin	3	0	28	20
Nitrate reduction to NO_2	86	37	86	80
Nitrate reduction to gas	68	4	10	7

* The *Agrobacterium*-like strains will be ascribed to a new genus within the *Rhizobiaceae* (Rüger & Höfle unpubl.)

strains, including the bacilli, were isolated on seawater agar at 2°C and all identification tests were performed in seawater media. Although not dependent on seawater base for growth, these strains are thought to contribute to the bacterial communities in this area.

Since every isolation procedure is highly selective, one cannot know whether we isolated members of the 'true' communities, i.e. those bacterial populations in the ecosystem which are not dormant, but metabolically active under the environmental and nutritional conditions given. During an earlier expedition to the northwest African upwelling region in 1975, we found bacterial communities different to those in 1982, but at that time we used an incubation temperature of 15°C for the enumeration and isolation of bacteria. *Agrobacterium*-like bacteria predominated in depths between 85 and 1023 m and members of the genus *Bacillus* in the depth range 1318 to 2998 m (Rüger 1985 and Table 3). These different results might be caused by changes in the environment during the period elapsed between the 2 expeditions or, more likely, by the selective effect of the different incubation temperatures applied.

No psychrophilic bacteria were isolated in 1975. Most of the *Agrobacterium*-like strains were not able to grow in freshwater media and are considered true marine bacteria, actively metabolizing at least in shallow stations, where sediment temperatures were higher than at deeper stations. At 4°C these organisms were not able to utilize numerous carbohydrates and N-compounds within 8 wk of incubation, but these substrates were utilized within 2 wk at 20°C (unpubl.). Only a few of the *Bacillus* strains isolated at 15°C in 1975 were able to grow at 5°C or required seawater media for growth, indicating that these organisms exhibited only low metabolic activities in the environment or even survived in a dormant state. These results were rather surprising, because the applied incubation temperature of 15°C did not differ greatly from the average temperatures found in the northwest African upwelling region (Table 2, Fütterer 1983). Moreover, the greatest proportion of the strains isolated at 2°C was also able to grow at 15°C (Fig. 1; Table 3) and one could postulate that these organisms could also have been isolated at this higher incubation temperature.

The proportion of strains capable of utilizing various carbohydrates, sugar alcohols and citrate was relatively high among the isolates from the area of low upwelling intensity, but decreased significantly in the central upwelling area. This might be connected with the observation that bacteria with an active respiratory electron transport system are less abundant in the Cape Blanc area than in the northern regions (Tan & Rüger 1989). Higher numbers of strains able to utilize various N-compounds and to reduce nitrates to nitrites, as found at 17/21°N, might be due to a maximum concentration of N-containing matter in this area of maximum upwelling intensity and a drastic decrease in the concentrations of these substrates north of it (Summerhayes 1983).

CONCLUSIONS

Any available method for studying marine bacterial communities by isolations of strains is selective in quality and quantity; the incubation temperatures applied for the isolation of bacteria are of especially great importance. In spite of these limitations, distinct benthic bacterial communitites were found in the centre of the northwest African upwelling area and a northern region low in upwelling intensity. The differences between these communities may be due the environmental and nutritional conditions in the area of investigation.

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