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ACTINOMYCETES OF THE BOTTOM SEDIMENTS OF VARIOUS SEAS

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ABSTRACT - Occurrence, distribution and physiological characteristics of actinomycetes in the top layer of the sediments of various seas were studied. Viable counts were very low in open sea sites. The majority of the isolates could be grouped to streptomycetes, Micromonosporae and nocardioforms. Streptomycetes are preponderantly distributed in coastal and shelf regions. They exhibit relatively broad temperature and salinity spectra and allow baroresistance. Micromonosporae are found in relation to the other taxa more frequently in deep sea sediments. They grow better in media with low salt concentration and have a temperature optimum around 30°C. On the other hand they are very baroresistant allowing these organisms to survive in the deep sea for long periods. Nocardioforms were found to form the major part of the sediment actinomycetes in open sea areas. Salt and monovalent cation dependence as well as a lower temperature optimum mark these organisms as indigenous bacteria. Since their maximal growth pressure is relatively low their distribution is limited up to about 2000 m. By numerical phenetic assay they could be distinguished from known nocardioforms and could be assigned to a new species of the genus *Rhodococcus : R.marinonascens*.

Key words : actinomycetes, bottom sediment, Atlantic Ocean, *Rhodococcus marinonascens*.

RÉSUMÉ - La fréquence, la distribution et les caractéristiques physiologiques des actinomycètes de la couche supérieure des sédiments ont été étudiées dans différents sites. En mer ouverte, le nombre d'actinomycètes viables est très faible. La majorité des isolats ont pu être regroupés dans les streptomycètes, Micromonosporae et nocardioformes. Les streptomycètes sont prépondérants dans les régions côtières et sur le plateau continental. Ils supportent une gamme de température et de salinité relativement large et sont barotolérants. Les Micromonosporae sont trouvés plus fréquemment dans les sédiments des eaux abyssales, en relation avec les autres taxons. Ils se développent mieux sur milieux à faible concentration en sels et leur température optimale est d'environ 30°C. Ils sont d'autre part très barotolérants, ce qui leur permet de survivre en milieu abyssal pendant de longues périodes. Les nocardioformes constituent la majeure partie des actinomycètes présents dans les sédiments des océans. Leur dépendance vis à vis des sels et des cations monovalents, aussi bien que leur basse température optimale les indiquent comme organismes indigènes. Puisque leur pression maximale de développement est relativement faible, leur distribution est limitée à environ 2000 m de profondeur. Par analyse numérique des caractères phénotypiques, ils ont pu être distingués des nocardioformes connus et classés dans une nouvelle espèce du genre *Rhodococcus : R. marinonascens*.

Mots clés : actinomycètes, sédiments, océan Atlantique, *Rhodococcus marinonascens*.

INTRODUCTION

Actinomycetes are usually not in the focus of marine microbiology. They are in many respects regarded as boundary bacteria and they are known as terrestrial organisms. Strains isolated from aquatic habitats are usually considered as wash-in organisms and, regarding longer periods, then living there in a dormant state (Cross T.1981). Isolations from marine areas are preponderantly reported from coastal or shelf regions. Only a few

surveys give us some knowledge about the occurrence in oceanic sites (Zobell CE. 1946, Kriss A.E. 1967, Weyland H. 1969, Walker J.D. and Collwell R.R. 1975, Okami Y. and Okazaki T. 1978, Weyland H. 1981 a).
In the course of our investigations on the bacterial populations of the sea sediments, actinomycetes could be found repeatedly. These observations gave rise to more detailed studies on occurrence, distribution, heterogeneity and activity status of actinomycetes in the marine environment.

MATERIAL AND METHODS

Sediment samples were collected during various cruises of the F.R.V."Anton Dohrn", R.V."Meteor" and R.V."Polarstern" using van Veen- and Shipek-grabs or Reineck-box corers. From immediately prepared dilutions of the top sediment layers pour plate and spread plate cultures were performed using a variety of media. In mangrove areas usually small boats and small van Veen-grabs were employed for sampling. In polar regions and in the Atlantic preliminaries and cultivations were performed at 1°C as well as at 18°C. Details of cultivation, counting and identification as well as of physiological tests : temperature characteristics, cation dependence, salinity requirement, barotolerance and baroresistance are discribed by H. Weyland (1981 a and 1981 b), by E. Helmke (1980 and 1981) and by E. Helmke and H.Weyland (1984).

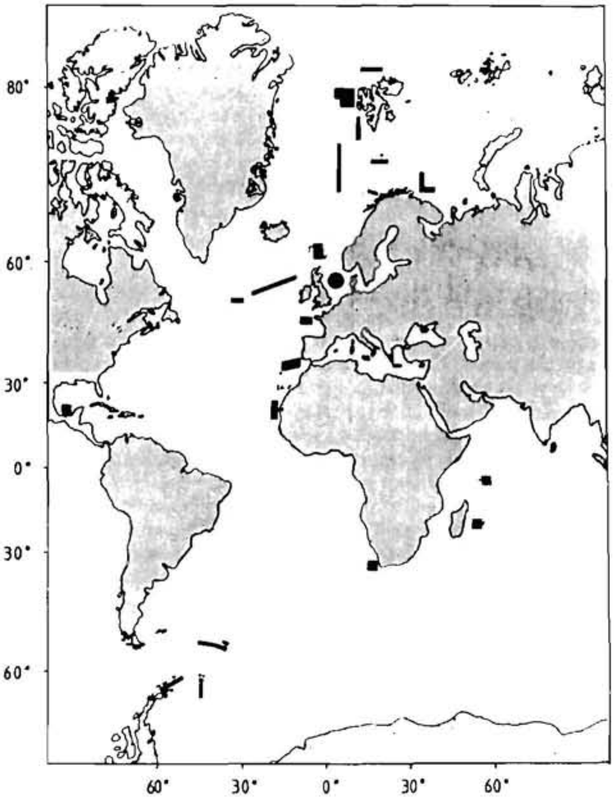

Figure 1 : Investigated areas.

RESULTS AND DISCUSSION

Various sea regions were investigated (Fig. 1). Results are given from areas of the Norwegian Sea north of the polar circle, off the Faeroe Islands, of the North Sea, of the North

Atlantic including an area of the Middle Atlantic Ridge, of the Biscay, of the Iberian Sea, of the upwelling zone off NW-Africa, of the Laguna de Terminos, Gulf of Mexico, and of the Antarctic Sea.

In table 1, some of these areas are quoted as illustrations of the amount of actinomycetes in relation to the total viable bacterial counts. Apart from the Antarctic Sea actinomycetes could be proved in the sediments of the majority of the stations of all areas. But in the open sea viable counts of the actinomycetes are usually low ranging about 100 cfu per ml wet sediment. They are indicated per thousand of the total viable counts. The mangroves exhibited the highest density of actinomycetes amongst the areas investigated also within their high salinity regions. In the sediments off NW-Africa the absolute numbers of actinomycetes per ml sediment are comparatively low in spite of the fact that this area is situated in a dust zone coming from the Sahara. Up till now actinomycetes could not be detected in the sediments of the Antarctic Sea. This may be due on the one hand to the absence of soil covered land and on the other hand to the Antarctic Ring Ocean which is seperated from the neighboured oceans.

depth m	sampling sites total	Act pos.	cfu/ml sediment (mean values) Bacteria	Act.	Act. V %
Norwegian Sea					
0-200	16	14	918 000	169	0.18
200-1000	41	40	394 000	298	0.8
1000-2000	14	14	106 000	164	1.54
> 2000	16	16	4 600	63	13.69
Antartic Sea					
0-200	2	0	18 743 000	0	0.0
200-1000	7	0	347 000	0	0.0
1000-2000	4	0	279 000	0	0.0
> 2000	12	0	162 000	0	0.0
North Sea					
0-200	38	34	1 637 000	480	0.29
200-1000	5	4	665 000	790	11.88
1000-2000	5	4	665 000	790	11.88
> 2000	5	4	665 000	790	11.88
Off NW-Africa					
0-200	10	6	246 000	46	0.19
200-1000	8	8	203 000	85	0.41
1000-2000	7	7	13 800	85	6.16
> 20008	8	8	4 800	46	9.58
Mangrove, Mexico					
0-10	10	10	10	16 651 000	397 000

Table 1 : Viable counts of bacteria and actinomycetes in the bottom sediments of different depth ranges.

Actinomycetes in general are a large bacterial group with several genera and a lot of species. The bulk of our isolates from the sea sediments could be classified only in three taxonomic groups : streptomycetes, Micromonosporae and nocardioforms. Isolates not belonging to these taxa only constituted a minority (Weyland H.1981 b).

Area	number of sampling sites	Streptomycetes per site	Micro monosporae per site	Nocardio forms per site	all strains per site
Norwegian Sea	83	0.9	3.4	5.5	9.8
North Atlantic	21	0.2	1.0	11.4	12.6
Off Faeroes	45	1.6	1.9	7.1	10.6
Biscay	47	0.5	7.2	2.1	9.8
Iberian Sea	27	1.8	3.6	0.6	6.0
Off NW-Africa	22	0.6	3.1	0.6	4.3
North Sea	106	4.4	4.6	0.7	9.7
Mangrove, Mexico	10	9.2	3.6	0.4	13.2
Antarctic sea	25	0.0	0.0	0.0	0.0
All regions	386	2.1	3.6	4.7	9.3
All region except North Sea, Antartic Sea and Mangrove	245	1.0	3.6	4.7	9.3
		11 %	39 %	50 %	100 %

Table 2 : Isolated strains of the major actinomycete groups. Mean values per site.

The isolation of the strains were performed in such a way that the number of differentiated isolates largely reflects the proportions of the different taxa present on the original culture plates. In table 2 the averaged values of the number of isolated strains of the three actinomycete taxa are given per site examined. Summing up the data of open sea areas investigated the nocardioforms were the most frequently isolated group (50 %),followed by the Micromonosporae (39 %). Streptomycetes have been found relatively seldom (11%). For example in the north Atlantic 5 sediment samples had to be examined in order to isolate one streptomycete strain whereas in one sediment sample 11 nocardioform strains were yielded. With regard to the frequency of the streptomycetes, the mangrove but also the North Sea are standing out.

Differences of the distribution of the three recognized taxa between the various areas become more obvious by comparing the relative proportions (Fig. 2). The northern areas, little effected by terrestrial run off and dust, exhibit a high proportion of nocardioforms. In other open sea areas remote from land the Micromonosporae predominate whereas in the North Sea and especially in the mangrove the streptomycetes are frequent.

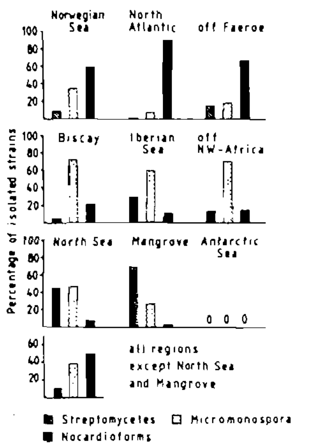

Figure 2 : Distribution of the major actinomycete groups of the various regions (in percent of the total number of actinomycetes isolated from one area).

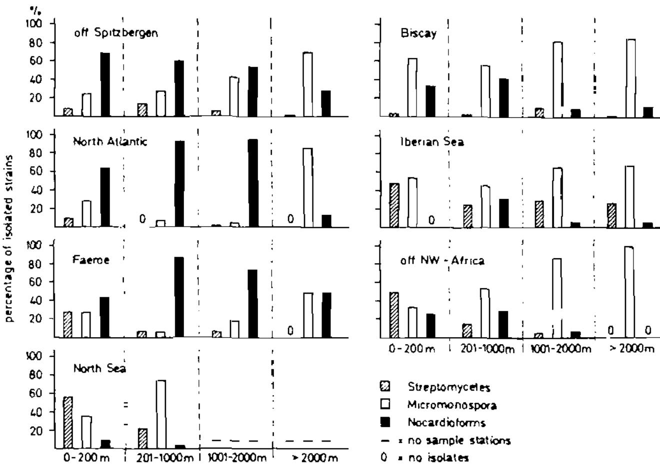

Figure 3 : Proportional distribution of the actinomycete groups dependent on depth. Based upon the ratios of isolates.

The sediments examined were hieved of course from different depths (Faeroe up to 2280 m, Norwegian Sea up to 3900 m, Atlantic up to 4062 m, Biscay up to 4700 m, Iberian Sea up to 5510 m, off NW-Africa up to 3362 m, North Sea up to 670 m, Antarctic Ocean up to 4419 m). When considering the distribution of the isolates of the three actinomycete groups in relation to various depth ranges - for each depth range the isolates of the groups are given in per cent - the following distribution pattern was found (arbitrary depth ranges) (Fig. 3) : High portions of Micromonosporae originated from deep sea sediments. An increase with depth of the relative amounts of the Micromonosporae can be stated. High or fairly high amounts of nocardioforms could be encountered in sediments of 200 to about 2000 m water depth. In the deepest range the relative amount of nocardioforms were found to be usually lower. The streptomycetes were represented by the sediments of shallow areas. Even in the North Sea, for example, a dominant position of the streptomycetes in sediment of the shallow range, was taken over by the Micromonosporae in the next deeper range (deep trench of the Skagerrak).

About the question, whether these distribution patterns are reflected by physiological characteristics, some studies were directed towards features that may indicate which group of these organisms is potentially able to metabolize and reproduce or can only survive in the marine environment.

The growth response to temperature of representatives of the three taxa are illustrated in figure.4, Streptomycetes and Micromonosporae showed temperature optima at 30°C or above 30°C. On the other hand the marine nocardioforms - in the meantime taxonomically affiliated to the genus *Rhodococcus* (Helmke E. and Weyland H. 1984) - do not grow at these temperatures. Their optimum is about 20°C. Considering the salinity requirement (Fig. 5) these rhodococci behaved like typical marine bacteria. They do not grow without salt. Media with concentrations of 75 % to 100 % seawater are optimal. Micromonosporae isolates are developing better in low sea salt concentrations. There is only low response to increased salt concentrations by streptomycetes. But the completion of their life cycle is favoured by the additions of some salt (Weyland H. 1981 b).

There was no significant effect of monovalent cations on the development of Micromonosporae as well as of streptomycetes. However the growth of the marine rhodococci depends on the addition of monovalent cations. This effect could not be observed with terrestrial *Rhodococcus*- and *Nocardia* spp. (Helmke E. 1980).

As a further criterion the barotolerance of the organisms were determined (Helmke E. 1981). The best barotolerance with respect to growth rate and yield were exhibited among the nocardioform strains. Growth rate and growth yields of marine as well as terrestrial strains decreased only a little up to the maximal growth pressure. The maximal growth pressure of the marine rhodococci were often relatively low. The Micromonosporae and streptomycetes examined were clearly more effected by pressures below their maximal growth pressure.

The pressure studies were supplemented with studies on the baroresistance in order to estimate the survival ability of the different actinomycete taxa above the maximal growth pressure (publication in preparation). The survival ability between these groups differed extremely. The nocardioform strains were killed rapidly by pressures exceeding the maximal growth pressure. Few of the streptomycetes tested survived high pressures with spores as the survival stages. These strains belonged to the yellow colour series. Spores as well as mycelium of the marine Micromonosporae survived long lasting treatments of high hydrostatic pressure at low temperatures. This baroresistance should permit Micromonosporae to survive deep sea conditions and it corresponds with the relative high isolation counts from deep sea sediments.

Summing up : the results of the physiological tests in connection with the distribution data give some indications on the potential abilities and possible role of actinomycetes in the sea.
The streptomycetes are most frequent in the coastal and shallow areas. The isolates usually have broad salinity and temperature spectra. Reproduction may be assumed in the shallow sites. In high salinity mangrove areas we found streptomycetes as the dominant actinomycete group.

The Micromonosporae were most frequent encountered in deep sea sediments. On the one hand, concerning temperature and salinity, they are not well adapted to marine conditions on the other hand, regarding baroresistance, they are furnished with high abilities to survive under deep sea conditions. Their occurrence in the deep sea sediment in a dormant status can be presumed.

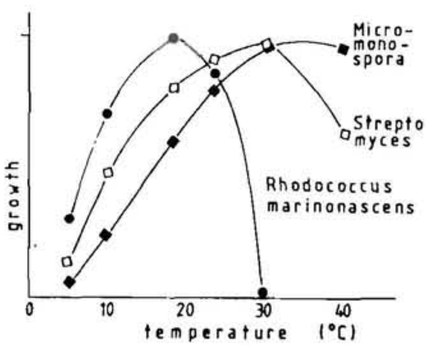

Figure 4 : Examples of growth response of the majority of streptomycete, Micromonosporae and Rhodococcus marinonascens isolates to temperature.

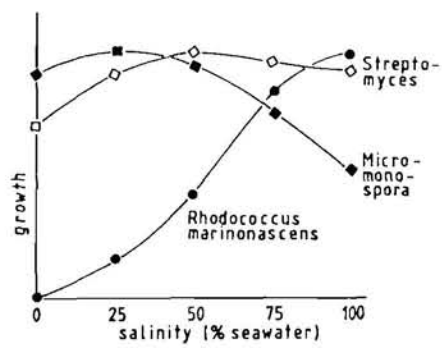

Figure 5 : Examples of growth response of the majority of streptomycete, Micromonosporae and Rhodococcus marinonascens isolates to salinity.

The non-sporeforming nocardioforms are found preponderantly in the open sea up to about 2000 m. Their survival ability at greater depth appeared to be relatively low. But they are best adapted for reproduction in marine environments up to moderate depths.

By a chemotaxonomic and numerical taxonomic assay the majority of the marine nocardioform isolates could be assigned to the genus *Rhodococcus* and clearly differentiated from other known species of that genus. These organisms could be given species status and referring to the exclusive occurrence of this taxon in the sea they were named *Rhodococcus marinonascens* (Helmke E. and Weyland H. 1984). By this organism an indigenous marine actinomycete could be made evident for the first time. Its niche is still unknown.

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