Actinomycetes in North Sea and Atlantic Ocean Sediments

THERE have been few reports of actinomycetes in the sea, and then usually only in the littoral zone and inshore localities[1]. While making extensive microbiological surveys Kriss *et al.*[2] found actinomycetes only occasionally and so it was assumed that these microorganisms are confined to the terrestrial habitat, and that those isolated from ocean sediments are forms not indigenous to the sea.

In 1966 we investigated the population density of heterotrophic bacteria in the water and the top layer of sediment of the Weser estuary and the German Bight. Actinomycetes were rarely observed on agar plates inoculated with water samples, but they were seen more often if bottom sediment was used, when plates were incubated for 4–6 weeks at 18° C. We later made a survey for the selective detection of actinomycetes in the bottom sediments of the North Sea.

During five cruises of the fisheries research vessel Anton Dohrn, between 1967 and February 1969, sediments were collected at 107 stations in various parts of the North Sea using van Veen and Shipek grabs. Material from the top 2 cm of sediment was immediately inoculated on pour plates with six of twelve previously tested culture media (Table 1).

Table 1. COMPOSITION OF MEDIA

Components	Medium designation SWA	DWA	CA	CHA	STNA	CZD
Peptone (g)	5	5	—	0·5	—	—
Peptone from casein (g)	—	—	1	—	—	—
Yeast extract (g)	1	1	—	0·1	—	—
KNO_3 (g)	—	—	—	—	3	—
$NaNO_3$ (g)	—	—	—	—	—	2
Saccharose (g)	—	—	—	—	—	15
Starch (g)	—	—	10	—	10	—
Chitin, hydrolysed and precipitated (g)	—	—	—	10	—	—
$FePO_4 \cdot H_2O$ (g)	0·01	0·01	0·01	0·01	0·01	0·01
Magnesium glycerophosphate (g)	—	—	—	—	—	0·5
Seawater (ml.)	750	—	750	750	750	750
Distilled water (ml.)	250	1,000	250	250	250	250
Agar (g)	15	15	15	15	15	15
*p*H	7·6 to 7·8	7·6 to 7·8	7·6 to 7·8	7·5	7·6 to 7·8	7·6 to 7·8

Of the 107 stations representing various types of sediment, 102 yielded colonies of actinomycetes in one or more types of culture medium. Actinomycete colonies derived from different samples often grew best on different culture media, suggesting that there is a variety of physiological strains or species.

There were 23–2,909 actinomycetes per cm^3. In coarse sand 30–69 m deep from the English Channel between Dover and the Isle of Wight were 92–1,485 per cm^3 (mean 510); in silt 435–690 m deep from the Skagerrak were 23–1,458 per cm^3 (mean 764); in various sediments 48–235 m deep from the central North Sea, including Devils Hole, were 23–115 per cm^3 (mean 54); in various sediments 76–164 m deep from the northern North Sea (transect Haugesund—Orkney Islands) were 23–230 per cm^3 (mean 128).

During a cruise of the research vessel Meteor in May 1968 similar results were obtained in the Atlantic Ocean off West Africa from 19° 00′ to 20° 40′ N. Sediment samples were taken from depths between 25 and 3,362 m and up to 175 nautical miles offshore. Nine out of twelve samples yielded actinomycetes although fewer colonies developed on the pour plates than when samples werefrom the North Sea. There were between twenty-three actinomycetes per cm^3 175 miles offshore at a depth of 3,362 m and 136 actinomycetes per cm^3 40 miles offshore at a depth of 299 m.

So far we have isolated 1,348 strains of actinomycetes from marine sediments. All pure cultures are being preserved in previously sterilized marine sediment for taxonomic investigation. The isolates include species of *Nocardia*, *Micromonospora*, *Microbispora* and *Streptomyces*. About half the strains develop aerial mycelia in pure culture.

In view of the high percentage of sediment samples that yielded actinomycetes and the fact that millilitre samples could be used for the detection of actinomycetes, these microorganisms in the sea seem to be neither random individuals nor temporary survivors of terrestrial run-off, but part of the marine ecosystem.

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Partial Inhibition by Mepacrine of the Development of Sulphonamide Resistance in *Plasmodium berghei*

RECENTLY interest has revived in the activity of the sulphonamides and sulphones against strains of malaria parasites, especially *P. falciparum*, that are resistant to other drugs such as chloroquine and pyrimethamine[1]. A number of acridine derivatives, including mepacrine, prevent the emergence *in vitro* of resistance to sulphonamides and certain antibiotics in *Staphylococcus* spp.[2,3]. Because mepacrine itself is a potential antimalarial blood schizontocide it seemed of interest to explore whether the simultaneous administration of mepacrine and a sulphonamide in malaria would result in any change in the pattern of development of resistance of the parasites to either drug.

The model selected was *P. berghei* (NK65 strain) in random-bred CFW mice free of *Eperythrozoon*. The first experiment was divided into two halves, A1 and B1, which were conducted side by side. In each half, three or four groups of five mice each were used for each passage. In each A1 passage one group of five animals served as untreated controls; the second group received a dose of sulphaphenazole known to be subcurative and the third a dose somewhat larger than this in solution or suspension. The drug was injected subcutaneously to all treated groups once daily. Passages were made by injecting intraperitoneally 10^7 infected erythrocytes from suitably diluted blood taken from a donor, this being the animal in which parasites of the previous passage had survived the highest dose of sulphaphenazole. Doses were adjusted so that, as far as possible, the erythrocyte infection rate (EIR) in at least a few treated animals (preferably in the group receiving the highest dose) reached 5 per cent or more by the end of 1 week. A fresh passage was made usually once a week. Occasionally it was necessary to stop drug treatment and delay the following passage until the EIR had built up to a sufficiently high level. The maximum dose of sulphaphenazole administrable by this method was 1,000 mg/kg; higher concentrations formed a suspension too thick to pass through a syringe needle. No drug toxicity was observed in the mice at this dosage.

Mice of the groups in B1 were similarly treated but, in addition, the animals which received therapy were given, as well as the sulphonamide, daily subcutaneous doses of mepacrine methanesulphonate in quantities from 1 to 10 mg/kg body weight. The maximum daily dose that did not significantly reduce the level of malaria parasitaemia by the end of a week compared with the untreated animals could be increased gradually from 1 to 6 mg/kg, but every attempt to increase this dose to 10 mg/kg resulted in almost total loss of the line and, indeed, I finally lost the B1 line.