SPHEROIDAL FORMS OF *BACILLUS MEGATERIUM* INDUCED BY POTASSIUM TELLURITE[1]

TOMIO KAWATA,[2] THEODORE SALL, AND STUART MUDD

Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

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This communication is concerned with the formation of spheroidal bodies from *Bacillus megaterium* strain KM, by the action of potassium tellurite in a hypertonic sucrose medium. Cells grown in sucrose-casein hydrolyzate broth (Lederberg and St. Clair, J. Bacteriol., **75,** 143, 1958) were inoculated into fresh broth and aerated at 37 C for 3 to 4 hr. Potassium tellurite (final concentration 0.05 per cent) was added to the cultures during the exponential growth phase. The cultures were placed in a candle jar and incubated under semianaerobic conditions for 18 hr at room temperature. Light microscopic examinations revealed many spheroidal bodies containing deposits of tellurium. With the electron microscope it may be seen that these deposits of tellurium are in the form of individual fine crystals peripherally disposed, and in clusters of crystals in local cytoplasmic sites contiguous to the plasma membrane. The significance of these observations will be discussed in a later paper by Mudd, Kawata, Payne, Sall, and Takagi.

In addition to spheroidal body formation from cells under growth conditions, washed resting cells, suspended in a 10 per cent sucrose-0.2 per cent $MgSO_4$ solution containing 0.05 per cent K_2TeO_4 and incubated at either room temperature or at 37 C under aerobic or anaerobic conditions, formed similar structures. The omission of sucrose from either the growth medium or a suspending medium (0.06 M phosphate buffer pH 7.3, 0.2 per cent $MgSO_4$, 0.05 per cent K_2TeO_4) resulted in almost complete lysis following 18 hr incubation under the conditions previously mentioned. Similarly, complete lysis occurred if suspensions of spheroidal bodies were diluted with either water or 0.06 M phosphate buffer (pH 7.3). It is interesting to note that the Robinow strain of *B. megaterium* did not behave in this manner. Our electron pictures do not reveal remnants of a cell wall on these spheroidal bodies, but lacking more definitive evidence of the absence of cell wall material, we refrain from referring to them as "protoplasts" (Brenner *et al.*, Nature, **181,** 1713, 1958).

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[2] Visiting Research Fellow. Present address: Department of Bacteriology, Tottori University School of Medicine, Yonago, Japan.

PRODUCTION OF ANTIBACTERIAL SUBSTANCES BY BENTHIC TROPICAL MARINE ALGAE

MARY BELLE ALLEN AND E. YALE DAWSON

Kaiser Foundation Research Institute, Richmond, California, and Beaudette Foundation for Biological Research, Solvang, California

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Recent studies have shown that planktonic marine algae may produce antibiotic substances that can account for the well-known bactericidal activity of sea water (Steeman-Nielsen, Papers Marine Biol. Oceanog. Deep Sea Research, **3,** (suppl.), 281, 1955; Sieburth and Burkholder, Abstr. of Comm. to Intern. Oceanog. Congr., 933, 1959). Methanol extracts of three benthic algae from Japanese waters, *Laminaria angustata*, *Undaria pinnatifida*, and *Rhodomela larix*, have been shown to inhibit several species of bacteria (Satio and Sameshima, J. Agr. Chem. Soc.,

TABLE 1

Inhibition of bacterial growth by methanol extracts of benthic marine algae

Sample No.	Alga	Inhibition of				
		Bacillus subtilis	*Staphylococcus aureus*	*Mycobacterium smegmatis*	*Escherichia coli*	*Pseudomonas aeruginosa*
7	*Cladophoropsis gracillima*	+	+	+	−	−
11	*Dictyota divaricata*	+	+	+	−	−
15	*Spyridia filamentosa*	+	+	±	−	−
16	Hydroid*	±	+	±	−	−
17	Hydroid†	±	+	±	−	−
20	*Gracilariopsis sjoestedtii*	±	+	±	−	−
22	*Enteromorpha prolifera*	+	+	+	−	−
23	*Enteromorpha compressa*	+	+	+	−	−
28	*Padina crispata*	+	±	−	−	−
28a	Hydroid‡	±	±	+	−	−
34	*Enteromorpha kylinii*	+	+	+	−	−
36	*Chnoospora minima*	±	±	−	−	−
38	*Chnoospora minima*	+	±	−	−	−
39	*Derbesia* sp. aff. *D. prolifica*	+	+	+	−	−

* With endozoic *Acrochaetium infestans*.

† With several endozoic and epizoic algae including *Acrochaetium infestans*, juvenile *Melobesia*, *Herposiphonia*, juvenile *Hypnea*, *Dictyota*, etc., several species of minute Cyanophyta including 2 species of *Lyngbya*.

‡ With minute epizoic and endozoic algae.

Japan, **29**, 427, 1955). These investigations suggest that production of antibiotics may be widespread in marine algae. This communication reports results obtained with a number of benthic tropical marine algae collected along the west coast of Central America and Mexico during March and April, 1959.

Algae were collected in intertidal and infratidal areas, frozen immediately after collection, and transported to the laboratory in a frozen condition. When samples of thalli were thawed and placed in cups on nutrient agar plates that had been seeded with representative bacteria, typical inhibitory zones resulted.

The antibacterial substance or substances could be extracted into water by incubation of the algal samples in distilled water at 37 C. Adding methanol to a final concentration of approximately 80 per cent permitted better extraction. The methanol was evaporated in a current of air at room temperature; the resulting aqueous solutions were neutral, and colorless to yellow. These were tested for antibacterial activity in the same manner as the thallus samples. Results are shown in table 1. No attempt has been made in this table to indicate the strength of the inhibitory response, since the concentration of the active materials is unknown, and the magnitude of inhibition by these crude extracts is somewhat variable. It will be seen that there is a wide distribution of materials inhibiting the growth of gram-positive bacteria, whereas gram-negative bacteria are unaffected by the algal extracts. The patterns of inhibition obtained suggest the presence of at least four different antibacterial substances in the algae studied.

Studies on the concentration and purification of the inhibitor material or materials are continuing.