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A Marine *Stichococcus* sp. which requires Vitamin B₁₂ (Cobalamin)

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SUMMARY: A marine *Stichococcus* sp. was found to require a growth factor present in small quantities in natural sea water. Probably the same factor was produced in the medium by a bacterium with which the alga was associated when isolated. The factor could be removed from sea water by activated charcoal, and could be replaced by crystalline vitamin B₁₂ (cobalamin).

The marine alga used in this investigation was collected in Great South Bay, Long Island, N.Y., by Mr J. H. Ryther (1954) and was purified as described below. There is some doubt as to the identity of the alga, which is a unicellular form about 2 μ . wide and 2-6 μ . long. It has been tentatively assigned by the present author to the genus *Stichococcus*, and may be identical with *S. cylindricus* Butcher (1952).

MATERIALS AND METHODS

Glassware. Pyrex test tubes, 15 cm., acid-cleaned, capped with glass vials.

Media. These were dispensed 5 ml./tube.

SW. Native sea water (North-west Arm, Halifax, N.S.; winter, 1952-3).

SWM. SW supplemented with minerals (g./l.): K₂HPO₄, 0.02; Ca(NO₃)₂·4H₂O, 0.10; traces of Fe, Mn, Mo, B, Cu, Zn (Burkholder & Nickell, 1949).

SSM. Artificial sea water (g./l.): NaCl, 23.0; MgCl₂, 5.0; Na₂SO₄, 4.0; NaHCO₃, 0.2; supplemented as under SWM.

Sterilization was by autoclaving at 15 lb./sq.in. for 15 min.

Other reagents. Activated charcoal (Norit-A; British Drug Houses Ltd., Toronto); Vitamin B₁₂ (crystalline, Merck).

Culture conditions. Constant illumination (220 f.c.) from white fluorescent tubes. Temperature, 23°.

Inoculation. From depleted SWM culture, with platinum loop.

Growth determinations. Optical densities of cell suspensions were made by means of a Klett densitometer, with ruby filter (No. 66). For cell counts a Fisher haemocytometer was used.

EXPERIMENTAL

Isolation of the alga

In the original culture the *Stichococcus* sp. was found to be associated with two other algae (*Chlorella* sp. and *Mischococcus* sp.) from which it was readily separated by streaking on SWM agar. By successive streakings it was possible

to eliminate all contaminants save one. The alga was apparently unable to grow well except in close association with bacterial colonies, suggesting that it might derive from the bacteria some organic micronutrient essential for growth. It was finally obtained in bacteria-free culture by streaking on SWM agar supplemented with Bacto-tryptone, and a pure clone (not necessarily identical with the pure culture independently isolated and studied by Ryther) was used in all subsequent tests.

Fig. 1. *a*: Effect of Norit-eluate on final yield of *Stichococcus* sp. in: A, native sea water, supplemented with mineral nutrients (SWM); C, Norit-treated sea water, supplemented as in A. One unit represents the amount of growth factor extracted from 1 ml. of sea water. *b*: Effect of tryptone on final yield of *Stichococcus* sp. Media as in 1*a*.

Identification of the growth factor

It was observed that the alga was capable of slight growth in SWM without further supplement, indicating that the sea water already contained small amounts of growth factor or factors. These traces could be removed by treating the sea water (1 l.) with activated charcoal (10 g.); and medium SWM, prepared with sea water so treated, did not support appreciable growth of the alga. Moreover, the factor could be eluted from the absorbent by ethanol. The charcoal was washed with 95% ethanol (50 ml.), the eluate evaporated to dryness on a water bath, and the residue taken up in medium SSM (10 ml.). The addition either of eluate or of Tryptone to charcoal-treated sea water again permitted growth of the alga (see Fig. 1).

A number of water-soluble vitamins were tested in charcoal-treated sea-water media, but only vitamin B₁₂ (cobalamin) proved effective in promoting the growth of the alga. The fact that growth could be obtained in a defined mineral salt mixture, SSM, supplemented by only vitamin B₁₂, indicated that no other growth factor was required (see Fig. 2).

Fig. 2. Effect of vitamin B₁₂ on final yield of *Stichococcus* sp. in: A, native sea water, supplemented with mineral nutrients (SWM); B, artificial sea water, supplemented as in A (SSM); C, Norit-treated sea water, supplemented as in A. In B and C, growth in the absence of added vitamin B₁₂ is attributable to carry-over of vitamin with the inoculum.

DISCUSSION

For maximum growth *c.* 0.2 μg. cobalamin/l. was required. Since vitamin B₁₂ is only partly destroyed by autoclaving (Robbins, Hervey & Stebbins, 1950), the error introduced when media were sterilized by heat does not seriously affect the values given here, which are merely indications of orders of magnitude. One cell apparently requires not less than 1.25×10^{-19} g. of vitamin B₁₂; or, taking the molecular weight of the vitamin as 1400,

$$\frac{6 \times 10^{23}}{1400} \times 1.25 \times 10^{-19} = c. 50 \text{ molecules.}$$

A cell of *Euglena gracilis* requires *c.* 4900 molecules of vitamin B₁₂ (Hutner, Provasoli, Schatz & Haskins, 1950): a cell of *Lobomonas rostrata* *c.* 5700 (Lewin, R. A., unpublished).

Assuming that the assay is quantitative, it may be deduced that the free soluble vitamin B₁₂ in sea water of the North-west Arm (Halifax, Nova Scotia)

was of the order of $0.01 \mu\text{g./l.}$ in the winter of 1952-3. This may be a higher value than would be found in water from the open sea, since the North-west Arm is a narrow inlet receiving considerable surface run-off from the city of Halifax and its environs.

REFERENCES

- BURKHOLDER, P. R. & NICKELL, L. G. (1949). Atypical growth of plants. I. Culture of virus tumors of *Rumex* on nutrient agar. *Bot. Gaz.* **110**, 426.
- BUTCHER, R. W. (1952). Contributions to our knowledge of the smaller marine algae. *J. Mar. biol. Ass. U.K.* **31**, 175.
- HUTNER, S. H., PROVASOLI, L., SCHATZ, A. & HASKINS, C. P. (1950). Some approaches to the study of the role of metals in the metabolism of micro-organisms. *Proc. Amer. phil. Soc.* **94**, 152.
- ROBBINS, W. J., HERVEY, A. & STEBBINS, M. E. (1950). Studies on *Euglena* and vitamin B₁₂. *Bull. Torrey bot. Cl.* **77**, 423.
- RYTHER, J. H. (1954). The ecology of phytoplankton blooms in Great South Bay and Moriches Bay, Long Island, New York. *Biol. Bull. Wood's Hole* (to be published).

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