ON THE ENRICHMENT AND SELECTION OF
BACTERIOCHLOROPHYLL - b BACTERIA FROM
THE BARNSTABLE MARSH - MASSACHUSETTS

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Abstract:

An attempt was made to determine if bacteriovorid species (BVD) containing bacteria are present in the Barnstable marsh near Woods Hole, Massachusetts, and to devise a method for the selection and enrichment of these bacteria. Results of both pigment extraction and the attempts to select for BVD-containing bacteria were negative. This indicates that BVD-containing bacteria are not present in the samples taken from Barnstable marsh.

Methods:

Extraction of pigments from soil samples:

Marine water was drained away from the soil which was subsequently washed twice with standard distilled water. Centrifugation was for about two minutes at 1000 x g. The lighter material at the top of the settled soil retained all of the original colored material of the soil. This lighter material was removed from the remaining grey soil at the bottom of the tube and resuspended in 1:10 volumes of 80% acetone: 20% distilled water. After incubation for 30 minutes, the sample was centrifuged at 1000 x g for five minutes. The spectrum of the supernatant was determined between 300 and 1000 nm using a PM 4 Hg Zin spectrophotometer and the
Selection for BCHL-β Bacteria:

A. Selection Media:

The two media used in the experiment were specifically designed to apply a selective pressure for photosynthetic bacteria and are as follows:

1. For Non-Sulfur Photosynthetic Bacteria: \( \text{NH}_4 \text{Cl} \)-Succinate

   - 0.01 M \( \text{NH}_4 \text{Cl} \) - Succinate
   - 0.1 M \( \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \)
   - 0.5 \( \text{K}_2 \text{HPO}_4 \) - Buffer, pH 7.0 at RT
   - 0.05 \( \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \)

   + Vitamins:
     - 250 mg/l Biotin
     - 250 mg/l Niacin
     - 250 mg/l Thiamin
     - 250 mg/l p- Aninobenzoate

   + Trace Metals:
     - 5 mg/l EDTA
     - 0.2 mg/l FeSO_4 \cdot 7\text{H}_2\text{O}
     - 0.1 mg/l ZnSO_4 \cdot 7\text{H}_2\text{O}
     - 0.03 mg/l MnCl_2 \cdot 4\text{H}_2\text{O}
     - 0.03 mg/l H_3BO_3
     - 0.03 mg/l Na_2 MoO_4 \cdot 2\text{H}_2\text{O}
     - 0.01 mg/l CuCl_2 \cdot 2\text{H}_2\text{O}

2. For Sulfur Photosynthetic Bacteria: \( \text{H}_2\text{S}/\text{NaHCO}_3 \)

   - 0.1% \( \text{H}_2\text{S} \)
   - 0.1% \( \text{NaHCO}_3 \)

   + Trace Metals (as above) + Sterile \text{N}_2 \text{S} to final 3 mM

   + Sterile \text{NaHCO}_3 to final 0.2% + Sterile \text{HCl} to final pH = 7.0
3. Anaerobic Methodology:

A soil sample from barestillic meadow was worked and dispersed in the basal media listed above. This suspension was used to inoculate a "shake dilution" series using the Na₂S/NaHCO₃ + 15% agar media in 12 ml test tubes. After solidification, the tubes were sealed with starch gauge wax.

This suspension was also used to inoculate three 125 ml "dilution bottles" designed to serve as "anaerobic petri dishes." These bottles were prepared as follows:

1. One bottle contained the (NH₄)₂-succinate media + 15% agar. The (NH₄)₂-succinate media + 15% agar was deoxygenated by strong boiling for five minutes, followed by cooling (24°C) while under a constant stream of deoxygenated argon. 25 ml of media was then dosed anaerobically into a 125 ml bottle previously flushed out with the same gas. The bottle was then closed with a butyl rubber cork and wired shut. After autoclaving, the bottle was laid on its side to permit the media to solidify along the first side of the bottle.

2. Two bottles contained the Na₂S/NaHCO₃ + 15% agar media. The basal media, trace metals and 15% agar were deoxygenated and autoclaved
in a round-bottom flask as per section 1 above. To this solution, sterile and deoxygenated \( \text{B}_{2}, \text{N}_{2}, \text{H}_{2} \text{O}, \text{H}_{2} \text{~S} \text{ and N}_2 \) were added. When maintained at 45° C, the media was asexually and aseptically dispensed into sterile bottles pre-flushed with deoxygenated argon. These bottles were also held on their sides.

Inoculation was performed under a constant stream of deoxygenated argon.

The "Light-Box":

Both the shake-stirrer series, and the "anoxic aerated shake" were placed in an aluminum lined cardboard box impervious to light, except through one 2 x 2 inch window. This window was blocked by an 80W warm incandescent light bulb plus the equivalent of a 1 cm gasketing of a 0.2% "copper-cyanide" solution. This solution was prepared by titrating a 0.2% CuSO₄ solution with concentrated H₂SO₄ until all of the cyanide was dissolved and the solution remained a bright "royal blue".

Results and Discussion:

The results of the pigment extraction are seen in Figure 1. The peak at 665 nm in an acetone extraction is indicative of the presence of BChl-a, while the lack of a peak at 793 nm in acetone indicates that there
is no Bchl-B present. The peak at 772 nm could be due to the presence of other Bchls (i.e. Bchl c, c₁, and c₂). In general, this method indicates that Bchl-B bacteria are either not present, or that they do not constitute a sufficiently large population to be detected against the large absorbance due to Bchl-a in this type of extraction. As seen below, the lack of Bchl-B bacteria seems to be the answer.

Under anaerobic conditions, the media described above should not provide an adequate energy supply for the growth and division of bacteria. In order to select for photosynthetic bacteria, light can be added as an "energy substrate" which is intelligible only by photosynthetic bacteria. Since bacteria containing Bchl-B can absorb and utilize light of longer wavelength than any other photosynthetic organisms, long-wavelength light should provide the selective pressure necessary to obtain Bchl-B bacteria from a mixed population of photosynthetic organisms.

Figure 2 shows the percent transmission of both the Wratten 87 filter and a 0.5% solution of "anirich-ammonium" of 1 cm pathlength. The resultant transmission of light is about 35-37% of the incident light. This light is also of pretty equal uniformity from 840-900 nm. When used in conjunction with the above specially modified media, bacteria containing
BChlA should have a selective advantage over other photosynthetic bacteria.

After four weeks in the "selection box," the only growth visible was small, colorless colonies on the NiCl₂/NaHCO₃ media and a few opaque colonies on the NiCl₂-succinate media. No pigmented colonies were observed anywhere. A possible reason for the appearance of growth under these conditions (other than photosynthetic bacteria) could be due to a slow fermentation by some organism of the contaminants found in commercial agar.

As a result of these two experiments, it appears that the sample obtained from Armatella mocki do not contain any BChlA-containing bacteria. Further experiments could be undertaken with a greater selection of samples and with the introduction of anti-fouling filters (not obtainable during this investigation) instead of the filters used herein.