Enrichment of "Chlorochromatium aggregatum".

Caroline Plugge,
Department of Microbiology,
H.van Suchtelenweg 4
6703 CT Wageningen.
THE NETHERLANDS
e-mail: Caroline.Plugge@algemeen.micr.wau.nl

Microbial Diversity Course
MBL, Woods Hole
July 1995.

Supervision: Dr.J.Overmann.

INTRODUCTION.

Among the green sulfur bacteria there are green and brown pigmented species. They can form different types of aggregates: star-shaped and net-shaped. Besides these aggregates there are also symbiotic associations described: Chlorochromatium aggregatum and Pelochromatium roseum (Schlegel,1993).
``Chlorochromatium aggregatum'' is a bacterial consortium which consists of two types of bacteria. One is an unidentified, slightly motile, sulfate reducing bacterium (SRB) and the other is a green sulfur bacterium. In the consortium the green sulfur bacterium is the ectosymbiont, loosely attached to the SRB. The metabolism of both organisms is linked, probably by the cycling of sulfate and sulfide: the sulfide produced by the SRB serves as an electrondonor for the green sulfur bacterium which results in the formation of sulfate; the sulfate is then used as an electron acceptor by the SRB. There is not much known about the characteristics of this consortium. Earlier studies showed that the ectosymbiont disappears after some time and the enrichment will slowly degenerate and eventually dies. The enrichment of this consortium is a delicate work and nobody has ever succeeded in isolating this consortium.
The aim of this study is to develop procedures to enrich for the ``Chlorochromatium aggregatum'' consortium.

MATERIALS AND METHODS.

Sources of inoculum.

Samples were collected at different times and spots from Oyster Pond (Woods Hole): close to the inlet and further up the pond as well as from School Street Marsh (Woods Hole). In both cases the top 5-10 cm of the sediment were sampled and transported to the laboratory in closed erlenmeyers or sampling cores. Part of the samples were overlaid with water from the sampling site and the others consisted of plain sediment.

Enrichments.

The first series of enrichments (7 erlenmeyers) all consisted of sediments overlaid with sampling site water. The erlenmeyers were placed in front of a fluorescent lamp and incubated at room temperature without further additions. Each day, samples were taken for microscopy.
A second series of samples (6 samples were collected) were taken from Oyster Pond. These enrichments were overlaid with prefiltered water from ``Bell Tower Pond''. After one day of incubation 10 mg/l of DCMU (3-(3,4 dichlorophenyl)-1,1-dimethylurea) was added to inhibit the anoxygenic photosynthesis. Some dithionite was added

to reduce the environment. Microscopic examinations were done daily.
A third series of samples (6 samples were collected) were taken at Oyster Pond. Two samples were overlaid with prefiltered water from ``Bell Tower Pond'', two with water from the sampling site and two with a 1:1 mixture of prefiltered water from ``Bell Tower Pond'' and spring water from the cafeteria in Swope. Sodium sulfide (0.1 mM) was added to reduce the flasks and all were placed in front of the light set. Daily microscopical observations were done.
Enrichments were subcultured in modified PNSB media which contained the following (per liter):
0.34 g KH_2PO_4; 0.34 g NH_4Cl; 0.34 g KCl; 0.5 g $MgSO_4$; 0.3 g $CaCl_2$. This solution was autoclaved and the following additions were made to finish the medium: $NaHCO_3$ 18 mM; $Na_2S.9H_2O$ 2.5 mM; 6-vitamin solution 1 ml; Trace element solution SL 12 1 ml. Final pH of the medium was set at 7.0 with HCl.
In some cases the medium was used at a 20% concentration with some modifications: $NaHCO_3$ 15 mM; $Na_2S.9H_2O$ 0.3 mM; Vitamin solution 0.5 ml and trace element solution 0.2 ml.
All incubations were done at room temperature.

Microscopy.

Routine light microscopy was carried out with a Zeiss phase-contrast microscope.
Pictures were taken with a Zeiss axioscope microscope using phase contrast and polarized light. Flagella were examined using DIC microscopy.

RESULTS AND DISCUSSION.

The first series of enrichments (7 erlenmeyers) all contained a wide variety of microbiota like Beggiatoa ssp.; Chromatium ssp; Thiovulum ssp.; several purple sulfur species; several green sulfur species and a lot of protozoa. But no ``Chlorochromatium'' could be observed. According to Overmann (personal communication) the microbial mat in Oyster Pond had a different look than previous years: there was a lot more activity of the ``pink organisms at the top of the mat''. This might explain the lack of ``Chlorochromatium'' in these enrichments. Also after 5-7 days no Chlorochromatium could be observed in one of the enrichments; neither from Oyster Pond, nor from School Street Marsh.
The second series of samples (6 erlenmeyers) again contained a wide variety of microbiota, similar to the ones observed in the first series. A decrease of the amount of species could be observed during time, due to the addition of DCMU. After 7 days of incubation no Chlorochromatium was observed.

The third series of samples (6 erlenmeyers) contained, again, a wide variety of microbiota in all enrichments. After 4 days Chlorochromatium could be observed in the enrichments with the 1:1 mixture of spring water and pond water. No Chlorochromatium could be observed in one of the other enrichments.
After 5 days, the amount of Chlorochromatium had increased even more. An explanation for the growth of Chlorochromatium in these enrichments might be that the amount of nutrients is too low for other bacteria to grow, and since Chlorochromatium requires a low nutrient level in order to grow.
Secondary enrichments were started by transferring 1 ml of the sediment surface into 30 ml sealed pyrex tubes with the modified PNSB medium at 100% at 20% strength. Parallel enrichments were started with 50 ml Pfennig bottles with 20% chromatium medium.
When samples from the third series were examined using polarized light, the ectosymbionts could be observed quite clearly See pictures in appendix). The amount of Chlorochromatium in comparison with the total amount of microbiota was estimated at 0.5-1%.
One remaining sample from the first series was also examined using polarized light. Also there some Chlorochromatium could be observed. The amount of Chlorochromatium in this sample was far less than 0.5% of the total population. The difference with the samples from the third series was, that the amount of ectosymbionts per consortium was around twice as much (See pictures). The consortia in this enrichment were already incubated for almost 3 weeks. This means that in this type of enrichment (sediment overlaid with sampling site water) the Chlorochromatium could be maintained, and slowly enriched.
An attempt was done to show the flagella of the consortium using DIC microscopy. The results were not so clear, because the consortium was floating through the sample all the time. The picture shows in two of the four pictures a vague curved flagellum. From the microscopical examinations one could see that the flagella were not attached to the ectosymbiont, but came from the central organism at the lateral side.

CONCLUSIONS.

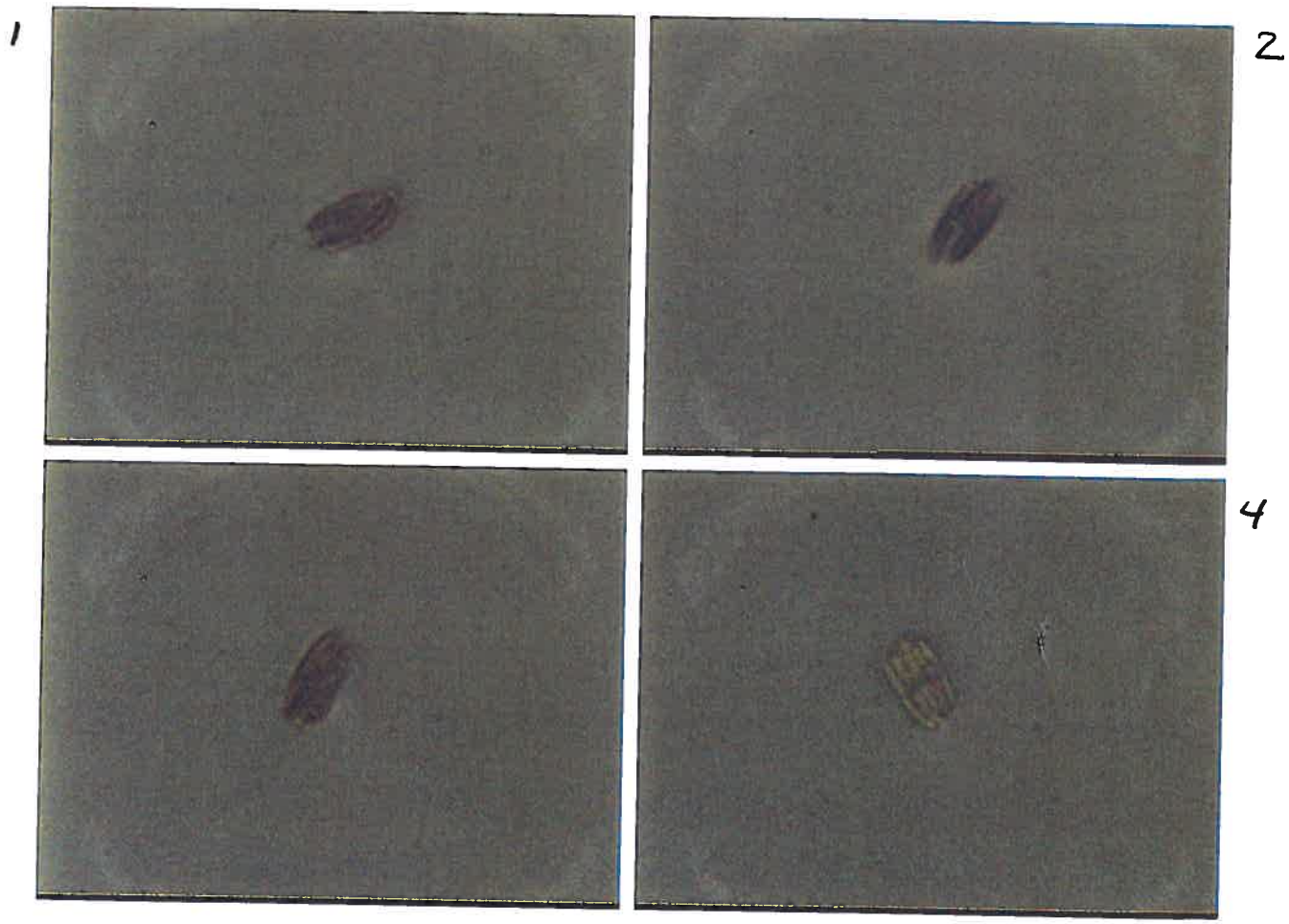
-''Chlorochromatium aggregatum'' can be enriched from Oyster Pond, using plain sediment overlaid with sampling site water or Bell tower pond water mixed (1:1) with cafeteria spring water.
-The amount of ectosymbionts per central cells in older enrichments increases.
-The flagellum of the consortium is probably attached at the central cell.

LITERATURE.

HG Schlegel (1993). Purple sulfur, non-sulfur and green sulfur bacteria. In: General Microbiology pp 410-411; seventh edition, Cambridge University Press.

P Egland (1993). Enrichment of ``Chlorochromatium aggregatum''. Report Microbial Diversity Course, MBL, Woods Hole.

Appendix



Polarized light picture of Chlorochromatium aggregatum from third series samples (1,2,3) and first series (4)

More pictures available in course files.

Thanks Tom.....!!!!