Study of the favorable conditions for the enrichment 
and eventual isolation of Spirochaeta plicatilis.

Magdalena Martinez-Canamero. Microbial Diversity Course. 
Marine Biological Laboratory. Woods Hole, MA 02543

Abstract

_Spirochaeta plicatilis_ is a microorganism known for more than a century but never isolated in pure culture, not even enriched, besides the natural primary enrichments. In this work, we have tried to settle the conditions which could make possible not only the mixed culture of this bacteria in the laboratory but also the eventual isolation, although this was never considered as a direct goal of the project. In order to do that, preliminary enrichments were designed following characteristics observed in the biology of _S. plicatilis_, as it is their association with tricomes of _Beggiatoa_ as well as with purple berries. When these enrichments didn't turn out successful, we tried to determine the basic conditions of light, temperature, pH and O₂. Once this was obtained, new enrichments were tried using the known information with two different concentrations of hydrogen sulfide and different carbon sources. Our results indicate that _S. plicatilis_ divides more actively at room temperature, in the dark, with a pH close to neutrality, low H₂S concentrations, complex carbon sources and very low O₂ or even anaerobic conditions.

Bibliography

Study of the favorable conditions for the enrichment and eventual isolation of Spirochaete plicatilis.

Magdalena Martinez-Canamero. Microbial Diversity Course.
Marine Biological Laboratory. Woods Hole, MA 02543

Abstract

Spirochaete plicatilis is a microorganism known for more than a century but never isolated in pure culture, not even enriched, besides the natural primary enrichments. In this work, we have tried to settle the conditions which could make possible not only the mixed culture of this bacteria in the laboratory but also the eventual isolation, although this was never considered as a direct goal of the project. In order to do that, preliminary enrichments were designed following characteristics observed in the biology of S. plicatilis, as it is their association with tricomes of Beggiatoa as well as with purple berries. When these enrichments didn't turn out successful, we tried to determine the basic conditions of light, temperature, pH and O₂. Once this was obtained, new enrichments were tried using the known information with two different concentrations of hydrogen sulfide and different carbon sources. Our results indicate that S. plicatilis divides more actively at room temperature, in the dark, with a pH close to neutrality, low H₂S concentrations, complex carbon sources and very low O₂ or even anaerobic conditions.

Introduction

Spirochaete plicatilis was first described in 1835 but it has never isolated in pure culture. It has been observed in fresh-water and marine, sulfide-containing environments, often in association with Beggiatoa tricomes. However, it has been considered the type species of the genus Spirochaeta although the wavy shape was the only spirochaeta-like feature which was really known in this bacterium for a long time. Only in 1973, Blakemore and Canale-Parola published a study using spirochetes from natural enrichments. This study covered the electron microscopy of the cells as well as their ecology. They were able to show outer sheath, axial fibrils and protoplasmic cylinder, proving that S.plicatilis was truly an spirochete. Their work also reports that the cells are 80-250 µm in length and 0.75 µm in diameter and they show a striking increase in number during the lysis of the Beggiatoa they are associated with. This information was our starting material.

Material and methods

Collection of samples.
Samples were taken from different freshwater and salt marshes and ponds as Cedar Swamp, School Street Swamp, Oyster Pond, Park Street Swamp and Sippewisset Marsh.
Media
Anaerobic mineral freshwater and marine medium was used for most of the enrichments, used under microaerobic conditions when necessary, and supplied in different occasions with methylcellulose, Y.E. 0.1%, PHB and βHB 0.2%, glucose 0.5%, triptone 0.1%. The enrichments were studied w/o rifampicine.

Cell counting
All the spirochetes present under the cover slip were counted on the optical microscope using the dark field on the lowest magnification. The total number was considered to occur in 5 μl of sample, and accordingly, the calculations of "spirochetes/ml" were made.

Results
We started collecting samples from Beggiatoa sulfur-containing mats where S. plicatilis was expected. It was found in two of these samples, from Oyster Pond and Sippewisset Marsh. These samples were used for some of the preliminary enrichments but soon they were depleted. However, most of the experiments were made using a second Sippewisset Marsh sample containing very few Beggiatoa and a relative large number of Spirulina instead. The following scheme synthesizes the first enrichments in which presence of Spirochetes could be noticed but never growth.

Mineral marine medium + red berries heat killed
Mineral marine medium + PHB
Mineral marine medium + rifampicine
Mineral marine medium + rifampicine
Mineral marine medium + rifampicine
Plain marine medium + rifampicine
Plain marine medium + rifampicine

Fig. 1
After the new sample was obtained, we tried to define the optimal temperature and light conditions for *S. plicatilis*. The results are shown in the following plot:

![Graph showing cell density under different conditions](image)

**Fig 2**

Therefore, the following enrichments were undertaken at R.T. and on the dark. The following figure shows:

A) the design of the next enrichments
B) the number of spirochetes after 24 and 48h.

A)

10 ml freshwater medium + 1 ml NaCl/MgCl₂

Set A

glass wool

3mM H₂S

10 ml freshwater medium + 1 ml NaCl/MgCl₂

Set B

glass wool

0.3mM H₂S

B/A + glucose 0.5%
B/A + βHB 0.2% 
B/A + glucose + βHB
B/A + triptone 0.1%

+/- Rifampicin

Inoculate with 0.25 ml from Sippewisset natural enrichment
Finally, the last data to be presented is the evolution of the original natural enrichment from Sippewissett Marsh during the whole extension of the research. As it will be discussed later, this gives us further information. It should be remarked that the enrichment was maintained in the light during the first week, in the dark the following four days and again in the dark the last three days.
Discussion

As we can observe in figure 2, the best conditions for the growth of *S. plicatilis* are dark conditions and room temperature. The original mat was kept in these conditions, that is why the number of cells is similar to that one in dark (this one was also kept at room temperature). With respect to the hydrogen sulfide concentrations, fig. 3B shows a clear preference for low concentrations. However, we believe that this presence is important, since H$_2$S can be measured in all the natural enrichments, as well as very low O$_2$ tensions.

The best carbon source turned out to be triptone, which allowed the number of spirochetes to double. However, after two days, only two of the samples with glucose increased the number. This factor should be further investigated. Finally, it seems that the best primary enrichment is the natural enrichment itself, always kept in the dark.

Our results enable us to establish certain primary conditions to begin an enrichment of *Spirochaeta plicatilis* in the laboratory. According to our experience, once a nice sample has been found from nature, the first step could be to keep the sample in conditions of dark and R.T. for around ten days. The number of spirochetes should be monitored. When the number is not raising anymore and the ratio with respect to other types of bacteria is decreasing, a new enrichment can be tried. Our results indicate that it would be a good idea to try a marine mineral medium at R.T., in the dark, with pH close to neutrality, low H$_2$S concentrations (0.3 mM) with complex carbon sources and/or glucose and under anaerobic or microaerobic conditions. A physical support as glass wool in the enrichments seems to be also a good idea.
Bibliography

Blakemore R.F. and E. Canale-Parola (1973) Arch.
Microbiology, 89, 273-289.