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# DISCUSSION OF THE BACILLUS FUNICULARIUS N.SP., AND A FEW REMARKS ABOUT THE GALLIONELLA FERRUGINEA EHRENBERG

by

A.J. Kluyver and C.B. van Niel
With 8 figures in the text

(From the Microbiology Laboratory, Technical University in Delft, Holland)

## 1. Introduction

Recently, diploma engineer A.P. Neeb isolated and preliminarily investigated in our laboratory a bacteria strain which, at least in one respect, seems to be worthy of further investigation.

The bacteria strain in question was found on a culture plate which had been prepared for the isolation of azotobacter chroococcum according to the classical Beijerinck's formula and which -- in addition to tap water agar -- contained 2% glucose, 2% whiting, and 0.1% $K_2HPO_4$. This plate was inoculated with a liquid culture medium which contained the same nutrients and which had been previously infected with garden earth and incubated at 30°C for two days.

For one reason or another, the expected azotobacter colonies did not develop on this plate. Instead, we found pure water-bright colonies of mucous consistence with an average diameter of 4 mm.

It is well known that the above-mentioned culture plates, which are prepared without the conscious addition of nitrogenous compounds, can be overgrown not only with true nitrogen-binding organisms, but occasionally also with numerous other microbes which are able to cover their nitrogen requirements from the small quantities of nitrogenous contaminants contained in the agar. Thus, there would have been no reason to pay much attention to the organisms found on our plate if the microscopic examination of the colonies had not revealed a remarkable picture.

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It turned out that we were dealing with an entangled fiber complex in which no natural fiber ends could be found with certainty. The occasionally observed ends made the impression of fractures which had occurred during sampling. It was equally strange that we were unable to disclose a cellular structure of the fibers. The diameter of the fibers was 1.0-1.8μ, with an average of 1.4μ. The mucous consistency of the colonies suggested that the fibers were covered with a distinct mucous sheath, and this presupposition was subsequently confirmed by microscopic examination of an india ink slide (See Figure 1).

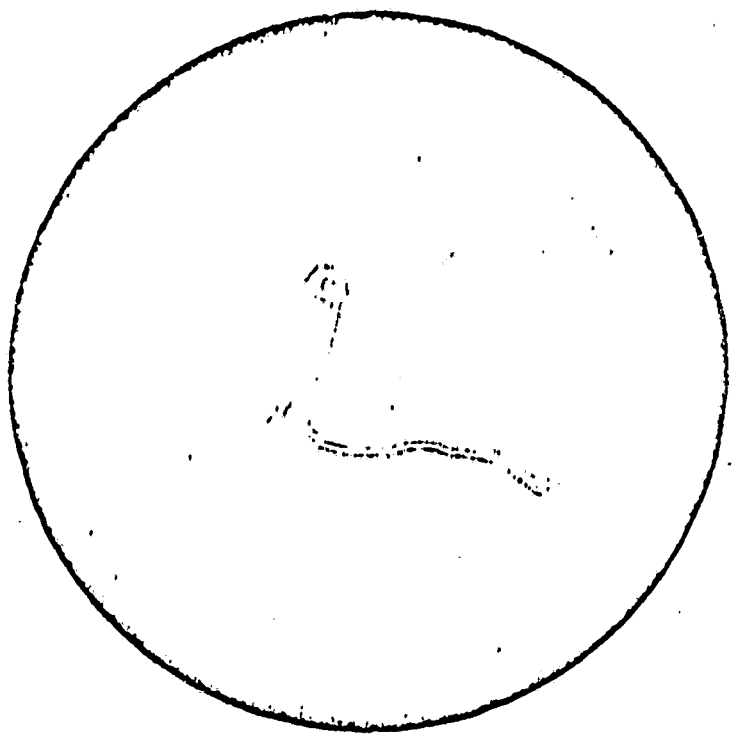

Figure 1

An india ink slide of a few fiber parts obtained from a facillus funicularius colony grown of yeast agar with 2% whiting and 2% glucose. The thick mucous sheath surrounding the actual bacteria fiber is clearly visible.

The rather strange morphological relations stimulated us to a closer investigation of this bacteria strain. For this purpose, we needed, in the first place, a pure culture of this organism. Because there was a high probability that the bacteria lacked the ability to bind elementary nitrogen -- and thus the addition of suitable nitrogen sources would not yield a better growth -- we continued our cultivation on an agar plate made of the usual yeast decoction to which 2%

glucose and 2% whiting had been added.

The fibrous structure of the original material made the preparation of a good suspension in sterile water rather difficult. Thus, inoculation of our culture plate with the suspension and subsequent cultivation at 30° C resulted in the development of only a few colonies. These, however, grew vigorously and reached a diameter of 1 cm and more in several days. The colonies were round and rather flat, and displayed a peculiar structure in so far as the central part of each colony was surrounded by a circular indentation followed, distally, by a wall-like elevation. A typical radial striation was observed on the surface of the colonies. When the culture dishes were opened, a faint and pleasant ester scent was perceived.

Figure 2. Fibers from the same colony as above, photographed in a dilute potassium iodide solution. Cellular fiber structure is clearly visible on places which are in focus (the fibers had not been fixed).

Microscopic examination of these colonies showed again a typical fiber complex. No cellular structure was observed in the fibers, and they were apparently filled with a homogenous protoplasm from which several fine kernels distinctly protruded at various places. The fact was clearly noticeable that several parts of the fiber complex were characterized by a more or less distinct spiral coiling of the fibers around each other. The presence of a thick mucous sheath was

demonstrated by means of india ink staining.

Since it is possible to demonstrate a cellular structure in the seemingly homogenous fibers of the fibrous iron bacteria through treatment with a potassium iodide solution, we decided to apply this treatment to our cultures. Successful results did not fail to appear: it became clearly apparent that the fibers were composed of numerous cells which were 4-6μ long (see Figure 2). The appearance of a deep red-brown color proved the presence of abundant glycogen.

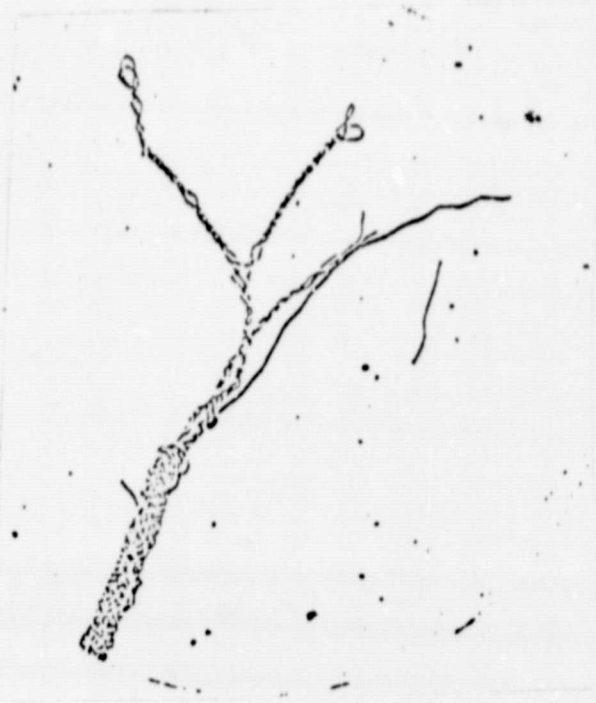

Figure 3. Typical bacteria cable from a culture of Bacillus funicularius grown in yeast decoction with 2% glucose. Formation of cables from simpler spiral tufts is clearly visible.

Aging changed the microscopic picture of the colonies developed in our culture medium very greatly. The fiber content soon changed into primarily an irregular, highly refractive, and more or less spherically shaped mass, while the cell walls usually dissolved and, finally, a typical cell detritus developed. We did not succeed in determining the chemical nature of the above-mentioned inclusion bodies. The usual fat reactions failed completely; only a small amount of fat was found on occasional places next to the above-mentioned bodies. The bodies were kept in concentrated sulfuric acid for several days and, strangely enough, they did not dissolve. Only a few fragments of a two-month old culture proved to be capable of growth. This circumstance has to be taken into account

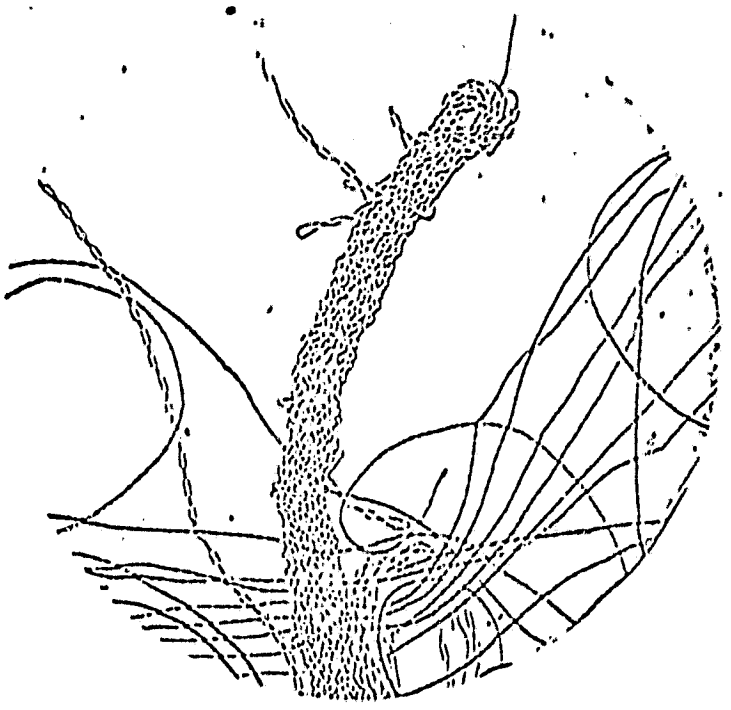

Figure 4. A second example of a typical bacteria cable of bacillus funicularius

Figure 5. A spiral tuft of bacillus funicularis at high magnification. It shows great similarity with Migula's pictures of gallionella ferruginea Enrenb.

when an old culture is used for inoculation and continuous cultivation.

In order to gain some insight into the physiology of this bacteria strain, which is rather unique in its morphological aspects, we decided to investigate whether the bacteria were able to develop under anaerobic conditions. For this purpose, a bottle was filled with a yeast decoction containing 2% glucose, the medium was inoculated with Neeb's pure culture,. and the bottle was plugged with a stopper. At the same time, a control culture was kept under aerobic conditions. It was found that no development, or only a very meager one, took place in the bottle while prolific growth was observed in the aerobic culture after a few days*. The liquid did not become opaque, but a rather large cotton-like flake

*It turned out to be very important to keep the hydrogen ion concentration low, so that the pH would range between 6 and 7.5. A simple method of doing this is to sterilize the liquid with chalk and to decant the clear supernatant fluid after the chalk has settled.

developed in the lower part of the culture medium. Microscopic examination of a part of this flake surprised us very much. It turned out that the flake consisted mostly of fibers spirally coiled around each other, and some of these coiled fibers were again coiled around each other, with the result that virtual cables were formed. Figures 3, 4, and 5 may give some insight into these structures. This strange formation stimulated us to further study.

2. Closer Investigation of the Isolated Bacteria Strain

It seemed important to investigate the behavior of the bacterium in other nutrient media. When samples of the pure culture, developed on Schreger agar of the above-mentioned composition, were transferred to strong culture media, it was repeatedly observed that growth occurred only on plates which obtained large samples. When this was taken into account, development was observed in wort agar, pea leaf agar, peptone agar, etc.

In wort agar, large, white -- later somewhat brownish -- colonies developed which, at first sight, were similar to those of various yeast types. A microscopic picture showed long fibers which were curved in a very irregular manner. This made them similar to the well-known picture of the lactobacetrium delbrücki culture grown on a strong substrate.

In pea leaf agar, white and flat colonies appeared, having a diameter of 3 mm. Slowly, a yellow field of diffusion would form around each colony. Microscopic examination of a young culture displayed nothing remarkable. However, when older colonies were studied in the microscope, we could observe in the fibers -- in addition to the irregular inclusion bodies occuring also in other nutrient media -- certain little bodies strongly resembling end spores.

The peptone agar culture dissipated all our doubts about the ability of the investigated bacteria strain to form spores. The 24-hour old colonies were transparent and showed a typical irridescence in transmitted light. Observation of these colonies under low magnification revealed a typical wavy structure which is well known for bacillus anthracis. Within three days, the colonies attained a

diameter of 3 mm, and they became white and opaque. Their microscopic examination showed that the fibers were filled with typical spores, as represented in Figure 6. In still older colonies, we could observe only a spore mass. The spores were relatively large, approximately 1.8μ long and 1.1μ wide.

Figure 6. Slide of a 5-day old culture grown in peptone agar. Spore formation is shown. Slide stained with simple methylene blue; spores remained unstained.

In order to prove that the inclusion bodies were spores, we subjected a suspension of an old colony to pasteurization at 80° C for the duration of 10 minutes. Smears of this suspension upon peptone agar yielded a prolific growth quite identical to that obtained from smears of a nonpasteurized suspension. When smears of the pasteurized suspension were made on yeast decoction agar containing 2% glucose and 2% chalk, the familiar and large colonies were obtained again.

These findings made it clear that the bacteria strain under investigation must have belonged to the bacillus genus (Lehmann and Neumann). According to our expectations, the bacillus yielded a Gram-positive reaction, while the peptone gelatin was slightly liquefied. However, motility was absent in all stages of development.

With respect to a diagnosis of the species, it was not possible to identify the bacterium with any one of the known species. That was improbable in any case, because the peculiar growth in liquid media -- a similar growth pattern was observed in 1% peptone water as in the yeast decoction with 2% glucose -- would have attracted the attention of investigators, and nothing could be found in the literature about this type of growth. For purposes of being doubly sure, we checked whether it was possible to identify the bacterium by means of the determination key given for the species of the bacillus genus in Bergey's Manual of Determinative Bacteriology.* It was not possible to do this we found.

Under these circumstances, it seems justifiable to us to regard the bacterium as a new type, from the view point of the literature. We should like to suggest for it the name, bacillus funicularius, because this name would express the most characteristic property of the organism -- namely, the formation of typical cables in liquid media.

### 3. The Mode of Formation of Tufts and Cables in Bacillus Funicularius

The growth of spiral tufts -- which is so characteristic for the bacillus funicularius -- has been known for a long period of time to be true of gallionella ferruginea and spirchaeta plicatilis. Migula(1) particularly called attention to the similarity in the growth pattern of these two organisms. A picture of spirochaeta plicatilis with spiral tufts can be found in Zopf(2). This author ascribed such growth forms also to a fiber portion of beggiatoa alba(3).

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(1) Ber. d.dtsch. botan. Ges., 15, 321, 1897, and Migula, W.: Bacteria Systems. Jena, 1031, 1897-1900.

(2) Zopf, W. Bacteria. Breslau, 1885. See Figure 4C. Also Magarete Zuelzer mentioned these tufts in her comprehensive treatise on spirochaeta plicatilis. Arch. f. Protistenkunde, 24,1, 1912.

(3) See Figure 1 by Zopf.

*Second Edition, Baltimore, 1926.

Fechner[1] reported on a similar formation of tufts in cyanophycee oscillatoria formosa.

In view of the fact that various authors give various explanations for the formation of tufts in the above-mentioned organisms, it seemed appropriate to perform a closer investigation of the well developed tufts in the easily grown bacillus funicularius. This was especially important because recently Cholodny has expressed revolutionary views about the morphology of one of the best known of the above-mentioned organisms -- namely, gallionella ferruginea. Temporarily leaving Cholodny's studies of gallionella embryology out of consideration (they will be thoroughly discussed in the next section), we can establish the fact that there are primarily two opinions about the mode of tuft formation in the above-mentioned microorganisms.

During Cholodny's time, most authors believed that the typical screw-like coiling of the gallionella ferruginea originated from a contact irritability which is characteristic for this organism. Stated Migula (loc. cit., page 325): "It is difficult to decide what is the cause of the peculiar behavior of the fibers in that they now form screws, now bend only slightly and grow without coiling around each other. Perhaps we should assume that the abvious tendency of the gallionella fibers toward a spiral growth is elicited also by contact stimuli. Even though curvatures can be observed in single fibers, these undulations are always irregular and never spiral-shaped. From the moment two fibers or fiber ends have touched each other, they never continue to grow in juxtaposition in straight or winding lines, but they immediately begin to form coils".

A similar view was expressed by Ellis in his book titled, "Iron Bacteria",

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[1] Fechner, R. Zeitschr. f. Botanik, 7, 289, 1915. See Figure 1, page 321.

as follows[1]: "A detailed investigation of this phenomenon is lacking, owing chiefly to the want of success that has attended efforts to obtain artificial cultures of Gallionella. Certain conclusions may, however, be arrived at based on comparisons with other organisms in which the same phenomena are apparent. There seems to be little doubt that Gallionella offers another example of contact irritability". On the other hand, special credit is due to Fechner for having directed our attention to a completely different explanation of the formation of spiral tufts in oscillatoria formosa. For the formation of tufts in the . . . . . (German text ends at this point).

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[1]Ellis, D. Iron Bacteria. London, 24, 1919.