THE GENERA LEUCOTHRIX AND THIOTHRIX

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We have isolated a remarkable filamentous marine organism, *Leucothrix mucor*, which was first described in 1844 by Oersted (1). *Leucothrix* may be characterized succinctly as a chemoheterotrophic counterpart of the colorless sulfur-oxidizing organism *Thiobrix*. It has been observed on a few occasions (2, 3) in the century since its original description, but the existing accounts of its morphology and development, based entirely on the examination of crude cultures, are either incomplete or inaccurate. Thanks to the ease with which it can be grown in pure culture, we have been able to determine its complete cycle of development, which includes a unique and hitherto undescribed process of gonidal aggregation to form many-celled rosettes. Such an aggregation stage has never been observed in *Thiobrix*, but the descriptions of this organism provide a good deal of circumstantial evidence for its occurrence, and we believe that the technical difficulties of cultivating *Thiobrix* have been responsible for the failure to detect it. Accordingly, we have decided to combine the description of *Leucothrix* with a critical review and reinterpretation of the scanty and scattered work on the morphology and development of *Thiobrix*.

**Enrichment and Isolation of Leucothrix**

A few years ago, one of us observed that marine spirilla can be regularly enriched by placing pieces of the thalli of marine algae in flasks almost filled with sea water and incubating these infusions for several days at 25 C. When a repetition of such enrichments was undertaken recently, we observed that some of the flasks contained long, colorless, immotile filaments with the characteristic cell structure of blue-green algae. Plates were being streaked at regular intervals from the infusions in order to isolate spirilla, and eventually colonies of the filamentous organism were discovered on one of these plates. It proved on further study to be *Leucothrix mucor*.

A repetition of the enrichment experiments was undertaken to discover, if possible, what factor determined the appearance of *Leucothrix*. A considerable number of infusions were prepared from algal thalli and incubated in diffuse daylight at 25 C. Seven species of algae were used, of which one belonged to the Chlorophyta, two to the Phaeophyta, and four to the Rhodophyta. Only infusions prepared from the green alga, *Ulva lactuca* Linn., gave rise to the development of *Leucothrix*, which appeared without fail in every flask containing *Ulva*. The enrichment procedure is, of course, far from specific. Within a few days a pellicle of bacteria and protozoa forms on the surface of the liquid, and the characteristic filaments of *Leucothrix* generally become evident upon microscopic examination of this pellicle about the eighth day. Between the eighth and twelfth days, *Leucothrix* has multiplied sufficiently to permit its isolation via streaked plates. It persists for a long time in such infusions; two strains were isolated from enrichment cultures almost 3 months old in which it was the dominant organism, its filaments forming a complex network throughout the culture.

For the isolation and maintenance of pure cultures we have used a dilute medium similar to that recommended by Pringsheim (4) for the cultivation of members of the Vitreoscillaceae. It consists of: tryptone 0.4 g, yeast extract 0.4 g, beef extract 0.2 g, Na acetate 0.2 g, synthetic sea water (5) 1 liter. After autoclaving, the pH is 8.0-8.3. For solid media, 2.0 per cent agar is added.

As a rule the colonies of *Leucothrix* comprise a small minority of those developing on plates streaked from enrichment flasks. However, the morphology is so characteristic that its colonies can be detected with ease when plates are examined with the aid of a dissecting microscope, and pure cultures can be readily obtained by picking young colonies from the first plates under the microscope and restreaking them. In all, 6 strains were isolated.

**General Description of Leucothrix**

In liquid cultures, *Leucothrix* grows in the form of long, colorless, unbranched, tapering threads which are attached basally to the wall of the cul-
ture vessel at the liquid-air interface and hang down into the medium. The basal diameter is about 3 μ and the apical diameter 1.5-2.0 μ. The length is extremely variable; it can attain well over 5 mm. Each filament consists of many cells with a smooth common outer wall. Occasionally individual cells in a filament die and autolyze with the production of empty compartments (the so-called "necridia" of the phycologist) that reveal very clearly the multicellular structure. A sheath cannot be detected, either by phase contrast microscopy of living filaments or by the examination of preparations suspended in nigrosin or India ink. The majority of the filaments in a liquid culture are arranged characteristically in the form of rosettes, consisting of many threads of varying lengths in close basal apposition. At the base of each rosette or individual filament is an inconspicuous holdfast. As a filament matures, the apical region develops a beaded appearance, caused by constriction of the outer wall at the transverse septa. This is followed by the abscission of single cells or short chains. After 24 hours, a liquid culture contains large numbers of such isolated cells, which show gliding motility in contact with a surface. The homologous structures in Thiothrix were designated as gonidia by Wino-gradsky (6), and we shall use the same term in this paper.

On solid media, very young colonies consist of a single filament (Plate I, figure 1). As it lengthens, it is thrown into regular folds to form a "thumbprint" colony (Plate I, figures 1 and 2). When examined under high magnification, the filaments show the typical apical-basal differentiation. Eventually the apical portions break up into gonidia, and older colonies consist as a result of a mixture of filaments and gonidia (Plate I, figures 3 and 4). The gonidia are immobilized and remain in chains at the sites of formation. They are, however, potentially motile, as can be shown by flooding a colony with liquid medium and observing the behavior of the gonidia through a cover-glass placed over the flooded colony. On dry agar surfaces, Leucothrix never forms rosettes. As will be explained in the next section, this is a consequence of the immobilization of the gonidia. Since the filaments themselves are permanently immobile, the extension of a colony on solid media is a purely passive phenomenon, and the colonies always remain compact and relatively restricted in size, seldom attaining diameters greater than 2.0 mm. The colony structure hence differs markedly from that of filamentous gliding organisms belonging to the family Vitreoscillaceae (7), which can spread rapidly over agar surfaces as a result of gliding movement.

All the strains studied proved to be very similar. On original isolation, they tended to fall into two groups which differed with respect to the length attained by the filaments prior to the formation of gonidia. In strains of one group the filaments broke up early, while in strains of the other group the formation of gonidia was delayed for a much longer period. This developmental difference was reflected by differences in colonial structure and in the macroscopic appearance of growth in liquid media. However, the distinction did not prove to be constant, and after maintenance for a month or two in pure culture all strains tend to reach a common condition, intermediate between the two extremes.

The Developmental Cycle of Leucothrix

The development of Leucothrix has been followed principally in slide cultures. These were prepared by sealing three edges of a coverslip to a slide with a vaseline-paraffin mixture so as to form a chamber of suitable depth and introducing a drop of inoculated medium from a capillary pipette through the open side, which was then immediately sealed to prevent evaporation. If a sufficient volume of air is included in the sealed preparation, growth proceeds normally for many hours, and the complete cycle of development can be observed under favorable optical conditions on undisturbed material.

We shall start the account of the life cycle with the liberation of gonidia from the apex of a mature filament, a process illustrated in Plate II, figures 5 to 7. When a filament matures, constrictions are formed between the terminal cells destined to be liberated as gonidia. Such a filament is shown in figure 5; the sixteen pre-gonidial terminal cells are clearly distinguishable. Just before the liberation of gonidia begins, the apex of the filament starts to wave back and forth in a rather jerky fashion. In figure 6 the eight terminal cells have broken off as a unit. In figure 7 the terminal portion of the filament has changed its position, and the chain of gonidia has started to glide away. As the gonidia break off and move away, maturation progresses down the filament, which continues to produce gonidia over a period...
of several hours. As a rule, the gonidia are liberated in short chains, rather than singly, but the chains mostly break up into individuals during their subsequent movements.

The gonidia are motile only in a liquid medium, and only in contact with a solid surface. Under these conditions, they show slow, short, jerky, interrupted movements, sometimes swinging back and forth while remaining attached to the substrate by one end of the cell. Flagella could not be demonstrated by staining. Evidently these cells are capable of gliding movement, similar to that shown by unicellular blue-green algae and myxobacteria.

Provided that large numbers of gonidia are concentrated over a relatively small area and that their motility is unimpeded, the next stage of the life cycle is gonidial aggregation to form rosettes. Plate III, figures 8 to 13 depict the course of gonidial aggregation in a single microscopic field over a period of 55 minutes. Three individual rosettes have been lettered in order to facilitate the comparison of successive photomicrographs.

In the earliest observable stage of aggregation (figure 9), three gonidia form a cluster in close apposition at one pole of the cell. Additional gonidia glide into the developing rosette until eventually a tightly packed structure is formed, the component cells radiating in all directions from the central point of attachment. Up to a certain point of development, aggregation is a reversible phenomenon, and rosettes containing as many as 12 individuals may redisperse completely. Larger rosettes appear to be incapable of disaggregation, probably as a result of the fact that some of the component cells have already become sessile. Secretion of a holdfast common to all the cells begins shortly after they have become sessile. The holdfast is a very inconspicuous structure, barely perceptible in living, unstained preparations. The best method which we have discovered for making it readily visible is to flood a slide culture with an aqueous 10 per cent (w/v) solution of nigrosine. The nigrosine is adsorbed by the holdfasts, which appear as black bodies in the center of the rosettes, contrasting well with the unstained cells. The holdfasts at the bases of isolated filaments are also clearly demonstrable by this technique (Plate IV, figure 14).

Each gonidium in a rosette grows into a multicellular filament, which continues to elongate until maturation occurs and a fresh crop of gonidia are liberated from the apical tip. Figure 15 shows a typical field of rosettes at an early stage of filament formation. The liberation of gonidia begins about 12 hours after rosette formation.

In order to observe aggregation uncomplicated by continued liberation of gonidia, slide cultures seeded with pure gonidial suspensions are necessary. Such suspensions may be obtained very simply by filtering a young liquid culture through Whatman no. 1 filter paper; as a result of their great length, the filaments are retained on the filter, and the filtrate consists almost exclusively of gonidia, which can be concentrated to any required density by centrifugation. Studies of slide cultures prepared from gonidial suspensions of different densities show that the initial density of the gonidial population is of cardinal importance in determining whether aggregation will occur. When the gonidia in a slide culture are separated from one another by an average distance of 60 μ or more, no rosettes are formed. Instead, each gonidium eventually settles down, develops an individual holdfast, and grows out into a single filament (figure 16). The gonidia are reproductive structures, and it is probable that the period during which active movement can occur is limited, just as in the case of a protistan zoospore. Consequently, a gonidium which does not manage to find its way into a rosette during this period is perforce condemned to isolated development. A second factor which may well operate to prevent rosette formation in dilute gonidial preparations is the weakness of the intercellular chemotactic gradients. It seems almost certain that chemotaxis plays a role in aggregation since chance collisions could never cause the pattern of accumulation shown in figures 8 to 13.

Since isolated gonidia can give rise to normal filaments which in time liberate gonidia again, it follows that rosette formation is not an obligatory feature of the life cycle. Furthermore, the fact that an isolated gonidium produces only one filament shows that rosettes cannot be formed by basal cell division in several planes; evidently, cell division is rigidly restricted to a single plane.

**Physiology of Leucothrix**

Since Leucothrix so closely resembles Thiothrix morphologically, the possibility existed that it might be a facultatively chemoheterotrophic
member of the *Thiothrix* group. Accordingly, washed filaments of *Leucothrix* were placed in sea water containing small amounts (approx. 1.5–150 mg per liter) of hydrogen sulfide and examined at intervals for the presence of intracellular sulfur globules. No evidence of sulfur accumulation was obtained. Our observations on enrichment cultures also make it somewhat improbable that *Leucothrix* is capable of oxidizing hydrogen sulfide. It has often been observed in algal infusions which also contained numerous *Beggiaota* and *Chromatium*, and although the cells of these organisms were packed with sulfur, the interior of the filaments of *Leucothrix* was at all times sulfur-free.

We have always grown *Leucothrix* in the complex medium described in the preceding section, but Dr. S. H. Hutner has very kindly examined its nutrient requirements for us, and he reports (personal communication) that it requires no growth factors, and can use a considerable variety of sugars and other simple organic compounds as sources of carbon and energy.

*Leucothrix* is a strict aerobe. The temperature maximum lies at 30°C, and the optimum at about 25°C. It grows best at a salt concentration (synthetic sea water) of 16 g per liter. Growth is still possible at a salt concentration of 3 g per liter, but the morphology is abnormal.

**Leucothrix, Chlamydothrix, Pontothrix: a Tangled Thread**

When we started our studies on *Leucothrix*, we recognized its close morphological similarity to *Thiothrix* but were unaware of any previous descriptions of chemoheterotrophic microorganisms possessing this peculiar structure. Hence, we believed that we had discovered a new organism; and since its physiological differences from *Thiothrix* clearly warranted generic segregation, it became our task to propose a name. *Leucothrix* seemed an apt choice, but on consulting Buchanan’s *General Systematic Bacteriology* (8) we found that it had been previously used. Buchanan’s account of its history follows:

“All *Leucothrix*. A generic name adopted from Oersted (1844) by De Toni and Trevisan (1889) to serve as a subgenus of *Leptotrichia* Trevisan. The type species is *Leptotrichia (Leucothrix) mucor* (Oersted) Trevisan. The subgeneric description given is simply “Marinae, majores.”

*Leucothrix* was previously used by Trevisan (1879) as a synonym in part of *Beggiaota*.”

This brief history aroused our curiosity, and after some difficulties we succeeded in obtaining a copy of Oersted’s opusculum, *De regionibus marinis, elementa topographiae historicanae* Oeresund. *Leucothrix* is described with classical terseness in the *enumeratio algarum viridium*, under the *Oscillatorinae*. Since the book is very rare,¹ we quote the original description:

*Leucothrix* mucor nob. in stagnis submarinis plantas filis suis radiantis mucorum modo obduct.

Character genericus. Fila alba et caespites et stratum, radii longissimis emissis, formatant, articuli distincti, interstitialis hyalinae simplicissime abaque constrictiobus, sporidia numerosa pulveracea alba.

Hoe genus a Calothrice, cui proximum est, et indole caespitis, valde radianti, et interstitiis multo distintioribus, ab omnibus confluentes sporidiorum indole valde recedit. Motiunculae ut apud ceteras Oscillatorinae interdum conspicue.

A translation² follows:

*Leucothrix* mucor nob. send out its radiating threads in marine pools much as molds do.

Generic characters. White threads forming both mats and layers, and sending out very long radiating strands, distinct articulations, with very simple unconstriicted hyaline interstices, sporidia numerous, powdery, white.

This genus may be separated from *Calothrix*, to which it is close, by the nature of the mats, which are clearly radiating, by its much more distinct interstices, and by the highly localized formation of sporidia. Motile sporidia are possible, as is sometimes observed in the other Oscillatorinae.

In this translation, *articulations* should be construed as *transverse walls, interstices as cells*, and *sporidia as hormogonia or gonidia*.

There can be no doubt that Oersted described an organism closely similar to, if not identical with, the organism which we have isolated: a

¹ Our copy was obtained from the Yale University Library.

² Kindly prepared for us by Dr. R. C. Baci-galupi, Curator of the Jepson Herbarium at the University of California.
MARINE COLORLESS BLUE-GREEN ALGA OF FILAMENTOUS STRUCTURE, SHOWING A RADIAL ARRANGEMENT OF VERY LONG THREADS. THE IDENTIFICATION RECEIVES FURTHER SUPPORT FROM OERSTED’S STATEMENT THAT LEUCOTHRIX IS SIMILAR TO CALOTHRIX, SINCE IT IS THE BLUE-GREEN ALGAE OF THIS GENERAL TYPE, NOW PLACED IN THE FAMILY RIVULARIACEAE, WHICH MOST CLOSELY RESEMBLE IN VEGETATIVE CONSTRUCTION THE MICROORGANISM ISOLATED BY US. THE ONLY OTHER KNOWN MICROORGANISM TO WHICH OERSTED’S DESCRIPTION COULD POSSIBLY BE APPLIED IS THIOTHRIX, BUT THE STATEMENT “HYALINE INTERSTICES (CELLS)” DOES NOT FIT, SINCE IN NATURE THE FILAMENTS OF THIOTHRIX ARE AS A RULE ALMOST OPAQUE ON ACCOUNT OF THEIR SULFUR CONTENT. HENCE WE ACCEPT OERSTED’S NAME FOR THE ORGANISM WHICH WE HAVE ISOLATED, AND WITH ALL THE MORE ALACRITY SINCE IT WAS OUR OWN CHOICE BEFORE WE KNEW OF HIS WORK.

WARNING,BY THIS EXPERIENCE, WE THEN CONDUCTED A MORE RIGOROUS SEARCH OF THE LITERATURE AND FOUND THAT LEUCOTHRIX HAD BEEN REPORTED ON TWO LATER OCCASIONS. IT WAS REDISCOVERED IN 1912 BY MOLISCH (2) WHO, IGNORANT OF OERSTED’S WORK, DESCRIBED IT BRIEFLY UNDER THE NAME OF CHLAMYDOTHRIX LONGISSIMA. IN 1932, NADSON AND KRASSILNIKOV (3) AMPLIFIED MOLISCH’S DESCRIPTION, AT THE SAME TIME CHANGING THE NAME TO PONTOTHRIX LONGISSIMA.

MOLISCH DESCRIBED CHLAMYDOTHRIX LONGISSIMA IN A PAPER PRINCIPALLY CONCERNED WITH MARINE SULFUR BACTERIA. THE RELEVANT PASSAGE IS TRANSLATED BELOW.

IN CONNECTION WITH THESE SULFUR BACTERIA A FILAMENTOUS BACTERIUM WILL BE DESCRIBED WHICH IS NOT A TRUE SULFUR BACTERIUM, BUT WHICH COMMONLY OCCURS WITH MARINE SULFUR BACTERIA AND IS A VERY CHARACTERISTIC COMPONENT OF THE MARINE SULFUR FLORA. IT IS SURPRISING THAT THIS ORGANISM, WITH ITS STRIKING SIZE, MASSIVE DEVELOPMENT AND FREQUENT OCCURRENCE, HAS NOT CAUGHT THE EYE OF BACTERIOLOGIST LONG AGO. I NAME THIS FILAMENTOUS BACTERIUM CHLAMYDOTHRIX LONGISSIMA MOLISCH.

IN ROTTING ALGAL INFUSIONS IN TRIESTE SEAWATER THERE FREQUENTLY OCCURRED ON THE SURFACE OF THE LIQUID A FILAMENTOUS BACTERIUM COMPOSED OF SHORT CYLINDRICAL CELLS. SULFUR IS NEVER DEPOSITED IN THE CELLS, AND ONLY DEPOSITED ON THEIR SURFACE WHEN THE THREADS LIE RIGHT AT THE AIR-WATER INTERFACE IN CONTACT WITH LARGE AMOUNTS OF OXYGEN. THE THREADS ARE COMMONLY FREE OF SULFUR, BOTH INTERNALLY AND EXTERNALLY.

THE THREADS RECALL A COLORLESS OSCILLARIA, BUT ARE IMMEDIATELY DISTINGUISHABLE BY THEIR IMPOTILITY. THEY ARE ALWAYS UNBRANCHED AND OF STRIKING LENGTH. THREADS 0.5 CM. OR MORE IN LENGTH ARE NOT UNCOMMON. IN MICROSCOPIC PREPARATIONS THEY LIE IN TANGLED MASSES, OR FORM ROPES COMPOSED OF NUMEROUS, OFTEN HUNDREDS, OF MORE OR LESS PARALLEL OR TWISTED SINGLE THREADS.

THE THREADS ARE NOT COMMONLY SURROUNDED BY A GELATINOUS SHEATH OF VARIABLE THICKNESS, WHICH IS NOT DIRECTLY VISIBLE AS A RULE. WHEN IT ATTAINS AN APPRECIABLE THICKNESS IT CAN BE SEEN IN WATER MOUNTS; AND BY THE USE OF INDIA INK, EVEN THE THINNER SHEATHS APPEAR AS A LIGHT BORDER AROUND THE FILAMENTS.

THE FILAMENTS WITHOUT SHEATH ARE 1-3 μ IN THICKNESS, FULLY FORMED FILAMENTS BEING ORDINARILY 2 μ THICK. THE FILAMENT CONSISTS OF CELLS AVERAGING 1-5 μ IN LENGTH, WHICH CAN BE DISTINGUISHED REASONABLY WELL FROM ONE ANOTHER EVEN IN LIVING MATERIAL.

THE THREADS ADHERE WITHOUT A DISTINCT HOLDFAST TO THE WATER SURFACE OR TO SMALL SOLID OBJECTS, AND FORM BUNCHES WHICH HANG DOWNWARDS.

THE YOUNG COLONIES SHOW A RADIATING ARRANGEMENT OF THE THREADS, SIMILAR TO THAT WHICH ONE OBSERVES IN THIOTHRIX. REPRODUCTION OCCURS BY DETACHMENT OF PORTIONS OF THE FILAMENT OR SINGLE CELLS. OCCASIONALLY A NOT VERY DISTINCT HOLDFAST CAN BE SEEN ON YOUNG GERMINATING FILAMENTS.

IN FIGURE 17 WE REPRODUCE MOLISCH’S DRAWING OF CHLAMYDOTHRIX LONGISSIMA. THIS ILLUSTRATION OF A YOUNG COLONY, WHICH SHOULD BE COMPARED TO OUR PHOTOGRAPH OF ROSETTES IN THE EARLY STAGES OF ELONGATION (FIGURE 15), SHOWS THAT HE WAS DEALING WITH THE SAME ORGANISM. THE ONLY FEATURE OF MOLISCH’S ACCOUNT WHICH DOES NOT TALLY WITH OUR FINDINGS IS THE DESCRIPTION OF A SHEATH. IN PURE CULTURE, LEUCOTHRIX SHOWS NO SIGN OF SHEATH FORMATION. THE DISCREPANCY MIGHT BE EXPLAINED IN TWO WAYS. IT IS POSSIBLE THAT UNDER CERTAIN CONDITIONS IN A NATURAL ENVIRONMENT, LEUCOTHRIX IS CAPABLE OF SHEATH FORMATION; OR ALTERNATIVELY, THAT IN ALGAL INFUSIONS IT OFTEN GROWS EMBEDDED IN ZOOGLEAL MASSES OF OTHER ORGANISMS, WHICH SIMULATE A GELATINOUS SHEATH AROUND THE LEUCOTHRIX THREADS. THE VARIABLE THICKNESS OF THE SHEATH AS DESCRIBED BY MOLISCH WOULD BE IN ACCORD WITH EITHER OF THESE INTERPRETATIONS.

THE DESCRIPTION OF THE SAME ORGANISM GIVEN IN 1932 BY NADSON AND KRASSILNIKOV (3) IS CONSIDERABLY MORE DETAILED THAN THAT OF MOLISCH, BUT CONTAINS A NUMBER OF FEATURES WHICH CANNOT BE RECONCILED AT ALL WITH OUR OBSERVATIONS. NADSON AND
Krassilnikov maintained the organism in crude culture for several months by successive transfer in algal infusions, but did not succeed in isolating pure cultures. Judging from their description and illustrations (reproduced in figure 18) it seems very likely that they confused other filamentous microorganisms, also present in the infusions, with developmental stages of Leucothrix. Their illustrations of mature sessile filaments and developing rosettes (figure 18: 3, 5, 10, 11), albeit crude, fit the picture for Leucothrix; but the peculiar thick cells occurring locally in normal filaments (figure 18: 1, 2) and the emergence of multicellular hormogonia of an oscillatory type from a sheath (figure 18: 3) do not. They failed to observe mobility of the gonidia and hence also missed the aggregation process.

Like Molisch, Nadson and Krassilnikov stressed the fact that even when growing in algal infusions richly populated by sulfur-filled filaments of Beggiatoa and Thiothrix, the threads of Leucothrix never contain sulfur.

Nadson and Krassilnikov's observations on the natural distribution of Leucothrix are of considerable interest. It was first seen by Nadson in 1898, growing in decaying algae on the shore of Heligoland. In 1914, he found it in a similar habitat at Naples, and in 1915 at Sevastopol. The study reported in 1932 was made on material again obtained from the Black Sea, and it was also observed at that time on algae collected at Alexandrovsk on the Polar Sea. Coupled with our observations and those of Molisch, this shows that Leucothrix is a widespread and common marine saprophyte in littoral regions, a fact which lends plausibility to the existence of a very early description in the algological literature, viz. that by Oersted.

This survey of the somewhat devious history of Leucothrix can be terminated most appropriately by an amended diagnosis.

_Leucothrix_ Oersted 1844 _emend._ Colorless immotile filaments up to 5 mm in length, tapering from base to apex, and commonly attached basally to solid substrates by an inconspicuous holdfast. Reproduction by means of gonidia, which are detached either singly or in small chains from the apical portion of the filament. The gonidia show gliding motility, and under favorable conditions aggregate to form rosettes containing up to 50 cells. The cells in rosettes become immotile, develop holdfasts, and elongate to form filaments. Hence mature filaments are characteristically arranged in the form of radial colonies. Gonidia can also develop singly, forming isolated filaments. Chemoheterotrophic.

**Synonym:** _Pontothrix_ Nadson and Krassilnikov, 1932.

One species, _Leucothrix mucor_ Oersted 1844 _emend._, with description as for genus.

**Synonyms:** _Chlamydothrix longissima_ Molisch 1912; _Pontothrix longissima_ Nadson and Krassilnikov 1932.

**Occurrence:** Widespread on decaying plant material in marine littoral regions.

The Morphology and Development of Thiothrix: a Review

The genus _Thiothrix_ was established by Winogradsky in 1888 (6) but is still very imperfectly known. During the course of time, seven species have been proposed; they are differentiated by the width of the filaments and by habitat. Van Niel (9) states: "the validity of these distinguishing characters is, however, doubtful because their constancy has not been sufficiently established; so far, the morphology of the _Thiothrix_ species has not been studied in pure cultures." The morphology and development of the various forms which have been described show a substantial uniformity, and accordingly in this review we shall attempt a general description and elucidation of the life cycle, without analyzing separately the data on each of the alleged "species".

The study of _Thiothrix_ is made peculiarly difficult by its growth requirements. So far as is known, it is an obligate chemoheterotroph which obtains its energy by the oxidation of H₂S. Hence its development is dependent on the presence of CO₂, O₂, and H₂S; furthermore, as Keil (10) has shown, the organism can tolerate only rather low concentrations of H₂S. This constellation of requirements adequately explains the fact that, with one dubious exception (10), pure cultures have never been obtained. The best available method for studying the development of _Thiothrix_ is microculture on slides, devised by Winogradsky (6).

Winogradsky's description of the morphology and life cycle of _Thiothrix_, still by far the most complete one, was based on the study of such microcultures. His conclusions are well summarized in his description of the new genus:
Non-motile filaments with transverse septa, surrounded by a delicate sheath, differentiated into an apical and a basal region, attached to solid objects by means of a sticky holdfast, filled with sulfur globules under normal conditions of existence. Reproduction by bacillus-form gonidia, endowed with slow gliding movement, which fix themselves to solid objects and elongate into filaments.

Figure 19 reproduces Winogradsky’s original drawing of Thiothrix.

The formation and behavior of the gonidia were described with great precision by Winogradsky, and since several later workers have failed to confirm his account, we will translate the relevant passage in its entirety.

When the filament attains a certain length—which is quite variable—one sees the initiation of the formation of gonidia. In a filament which is still young and not more than 100 μ in length, the process occurs in the simplest fashion: the terminal portion, 8-10 μ long, disarticulates from the parent filament, remaining attached only by the common sheath. At the same time, it starts to show motility, which is no more than a slight trembling; but soon the movements become swinging, the terminal portion making an angle with the filament, or even coming to rest alongside it. All movements are quite slow, and interrupted by long pauses. At a certain moment the motile terminal portion attaches itself to the coverslip, as shown by the fact that it no longer responds to slight jarring movements which make the parent filament tremble. It then moves away in gliding fashion, drawing after it the floating portion of the parent filament until tension breaks the connection, and the filament retracts like a taut spring. Movement lasts only 1-3 hours, during which time the motile gonidium covers a distance of only 50-100 μ. The movements are slow and irregular; sometimes it glides with its full length on the substrate, sometimes it stands on end, but always remaining fixed to the glass. It finally comes to a halt and starts to elongate.

The motility of the gonidia was confirmed by one later observer, Wille (11), but since he misinterpreted the intracellular sulfur globules as gas vacuoles, he can scarcely be considered a reliable witness. Keil (10), the only worker after Winogradsky to explore in detail the cultivation and physiology of Thiothrix, failed to observe active movement despite a careful search, and Pringsheim (4) was equally unsuccessful. Neither Keil nor Pringsheim denied the possibility that such movement does exist, and indeed a denial could scarcely be entertained in the face of Winogradsky’s highly circumstantial account. As Pringsheim (4) says: “the capacity for gliding movement is easily disturbed by changed conditions, and the settlement of young filaments is scarcely explicable without motile reproductive stages.”

In our opinion, the failure of later observers to confirm this feature of Winogradsky’s observations simply reflects the extreme difficulty of maintaining slide cultures of Thiothrix continuously in a favorable physiological state.

In one crucial respect, Winogradsky’s description of the morphology of Thiothrix in microcultures deviates markedly from descriptions based on the examination of natural material. In crude cultures, as he himself states, Thiothrix forms radial colonies, the filaments pointing out in all directions from a central zone of attachment. However, there is no evidence that he observed well formed radial colonies in microcultures. The characteristic natural mature growth habit of Thiothrix is beautifully portrayed in Miyoshi’s (12) drawing (figure 20), and in a recent photograph, kindly lent to us by Dr. E. J. Ordal (Plate VI, figure 21). The only illustrations of the early development of these radial colonies which we have discovered are those of Molisch (2); their resemblance to young radial colonies of Leucothrix is striking. Molisch makes a statement about these colonies which suggests that he may have suspected that an aggregation process preceded their formation: “die jungen Keimlinge pfliegen nesterweise beisammen zu sitzen und wachsen strahlenartig aus.”

In effect, however, none of the students of Thiothrix has attempted to explain the characteristic radial growth habit, and the curious developmental problem which it poses does not seem to have been clearly appreciated. Since Winogradsky’s observations show that the gonidia achieve a certain dispersion, only two mechanisms can be entertained to account for the ultimately observed radial growth habit. One is the occurrence of a mode of cell division (e.g., basal budding or fission in several planes) during the early stages of development that is radically different from the regular divisions in a single plane occurring later during elongation of the filaments. The other is gonidal aggregation. In view of our observations on Leucothrix, it may be asserted with confidence that the latter mechanism is the one operative in
Thiothrix. It remains, however, to explain why gonidial aggregation in Thiothrix has never been observed. Since most students of the group after Winogradsky could not even detect gonidial movement, their failure is readily understandable. Winogradsky, however, examined preparations in which the gonidia were motile. It should be recalled that Leucothrix does not form rosettes when the gonidia are widely separated from one another; under these conditions they settle down singly and grow into isolated filaments which closely resemble the filaments of Thiothrix figured by Winogradsky. Hence his failure to see aggregation was probably a consequence of the fact that the gonidia in his preparations never occurred with the requisite abundance.

To summarize the conclusions reached from this review of the literature on Thiothrix, we are of the opinion that Thiothrix is characterized by a cycle of development in all respects identical with that of Leucothrix but that the existing descriptions (even that of Winogradsky) are incomplete, gonidial aggregation having been completely overlooked as a result of the difficulties inherent in the study of an obligate chemoheterotroph with the particular requirements shown by Leucothrix.

The Systematic Position of Leucothrix and Thiothrix

Leucothrix and Thiothrix clearly belong among the colorless, filamentous, gliding organisms, which include also the Beggiatoaceae sensu Pringsheim (4) and the Vitreoscillaeae (7). In his mastery of the relationships between bacteria and blue-green algae, Pringsheim (4) had already recognized the important morphological differences between Thiothrix and Beggiatoa and Thiotricha, the two other groups of filamentous sulfur bacteria with which it had been associated in the family Beggiatoaceae, and he placed it in a separate family, the Thiotrichaceae. We fully concur with the systematic judgment implied by this proposal. Thiothrix and Leucothrix stand apart from all other filamentous, gliding bacteria as much more highly differentiated organisms. This is shown by the functional and structural polarization of the filament; by the restriction of motility to specialized reproductive cells; and above all, by the occurrence of an aggregation stage. In fact, Leucothrix and Thiothrix rank with the fruiting myxobacteria and the higher actinomycetes as the most complex of bacteria.

It is well known that the filamentous, gliding bacteria show close morphological affinities with filamentous blue-green algae, and that many of them probably represent, phylogenetically speaking, apochlorotic descendants of the Myxophyta. The evidence on which this conclusion rests has been analyzed recently in great detail by Pringsheim (4), and we will not recapitulate it here. The representatives of the Beggiatoaceae and Vitreoscillaeae find their photosynthetic counterparts in blue-green algae of the family Oscillatoriaceae; Beggiatoa itself is structurally indistinguishable from an Oscillatoria, apart from the presence of sulfur inclusions. On the assumption that an analogous derivation may have occurred in the case of Leucothrix and Thiothrix, we shall now consider to what existing groups of blue-green algae, if any, they show affinities. A close relationship to members of the Oscillatoriaceae appears most improbable since these blue-green algae do not show a marked apical-basal differentiation of the filament. Furthermore, the reproductive structures of Leucothrix and Thiothrix are quite different from the hormogonia of the Oscillatoriaceae. A hormogonium is a short filament of cells which, as stressed by Geitler (13), behaves both morphologically and physiologically as a unit; unicellular, motile hormogonia have not been described with certainty. It is for this reason that we chose to employ for the reproductive cells of Leucothrix and Thiothrix the regrettably ill defined term gonidium, in preference to the well defined but inapplicable term hormogonia. In order to make perfectly clear the distinction between these two kinds of reproductive structures, and also to confer a much-needed precision on the term gonidium, we shall redefine it as follows. A gonidium is a unicellular reproductive structure capable of gliding movement which is produced by abscission from the terminal portion of a multicellular filament.

Sessile filaments with a pronounced apical-basal differentiation are found most characteristically among blue-green algae of the family Rivulariaceae. Furthermore, many members of the Rivulariaceae share to a greater or lesser extent the characteristic growth habit of Leucothrix and Thiothrix, producing tufts or radially-arranged groups of filaments. Indeed Oersted already drew attention in his original description of Leucothrix to its structural similarities to Calothrix, a blue-green alga now placed in the...
Rivulariaceae. There are, however, several features of *Leucothrix* and *Thiothrix* which do not fit very well the postulate that they are apochlorotic relatives of the Rivulariaceae. The first is the absence of a sheath. Most (all?) members of the Rivulariaceae have a definite sheath. Secondly, the filaments in the Rivulariaceae are usually terminated basally by a specialized cell, known as a heterocyst; this feature is lacking, however, in *Homeothrix* and *Ammatoidea*. *Homeothrix* further resembles *Leucothrix* and *Thiothrix* in that it does not show false branching, which is otherwise widespread in the Rivulariaceae. But despite these resemblances, there is one major objection to regarding *Leucothrix* and *Thiothrix* as apochlorotic relatives of rivularian forms like *Homeothrix*; namely, the apparent absence in the Rivulariaceae (and indeed in all other filamentous blue-green algae) of reproductive structures homologous with gonidia, which can undergo aggregation. It remains possible that such features may be found in blue-green algae of a rivularian type since these organisms have been described for the most part in superficial fashion on the basis of observations on natural material, and an aggregation stage, if it existed, could be overlooked very easily under these conditions, as the early history of *Leucothrix* shows. The habit of the colony, particularly in unbranched forms like *Homeothrix*, is at least suggestive of the possibility that an overlooked aggregation stage may exist. Were such to be found, the systematic position of *Leucothrix* and *Thiothrix* could be considered as settled; but for the time being, it is far from clear.

In conclusion, two other systematic proposals concerning *Leucothrix* and *Thiothrix* should be mentioned, if only to be dismissed. Peshkoff (14) included Nadson and Krassilnikov’s *Pontothrix longissima* (which is identical with *Leucothrix mucor*) in the order Caryophanales, whose best studied representative is *Caryophanon latum*. *Caryophanon* is a multicellular, filamentous bacterium which is motile by means of peritrichous flagella and reproduces by binary fission of the filament. Peshkoff (15) regarded it as intermediate between bacteria and blue-green algae, but as Pringsheim (4) pointed out, there is no real basis for this contention and it is, in fact, a typical if complex eubacterial form. Hence *Leucothrix* (*Pontothrix*) has no place in an order of which *Caryophanon* is the representative type.

*Leucothrix* (Chlamydothrix) was included in the Chlamydobacteriaceae by Molisch (2), and a similar position for *Thiothrix* was recently mooted by Bisset and Grace (16), who incorrectly assumed that the gonidia are immotile. The best studied members of the Chlamydobacteriaceae are filamentous organisms of the *Leptothrix-Sphaerotilus* type (17) which reproduce by means of flagellated swarers (see Stokes (18) for superb illustrations of their structure). They are thus eubacterial forms, albeit quite different in construction and development from filamentous eubacteria of the *Caryophanon* type. The grounds for assuming a relationship of *Leucothrix* and *Thiothrix* to these bacteria are no stronger than the grounds for assuming a relationship to *Caryophanon*.

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Plate II, figs. 5-7. *Leucothrix mucor*. Separation of gonidia from the tip of a mature filament. Consecutive photographs of a single filament taken over a period of 7 minutes. Dark phase contrast, X 750. (Enlargement, 1.9 X).
PLATE III, figs. 8-13. Leucothrix mucor. The aggregation of gonidia to form rosettes. Consecutive photographs of a single microscopic field, taken over a period of 55 minutes. In figs. 9-13, three developing rosettes have been lettered in order to facilitate comparison. Dark phase contrast, X 500. (Enlargement, 1.28 X).
Plate IV, figs. 14–16. 

**Leucothrix mucor.** Fig. 14. Nigrosine mount from a liquid culture 12 hours old, showing the holdfast (dark area) at the base of an isolated filament, $\times 1,500$. (Enlargement, 2.22 $\times$). Fig. 15. A field of young developing rosettes, wet mount in 0.1% methylene blue. $\times 1,500$. (Enlargement, 3 $\times$). Fig. 16. A field of isolated filaments, developing in a slide culture from gonida too widely separated from one another to permit aggregation. Wet mount in 0.1% methylene blue. $\times 400$. (Enlargement, 1.68 $\times$).
Plate V, figs. 17-20. Illustrations of Leucothrix and Thiothrix taken from earlier publications. Fig. 17. Habit sketch of young rosettes of Chlamydothrix longissima (= Leucothrix mucor), reproduced from Molisch (2). Fig. 18. Drawings of various stages of the life cycle of Pontothrix longissima (= Leucothrix mucor), reproduced from Nadson and Krassilnikov (3). Fig. 19. Filaments of Thiothrix growing in a slide culture, reproduced from Winogradsky (6). Fig. 20. Habit sketch of Thiothrix growing in nature, reproduced from Miyoshi (12).
PLATE VI, fig. 21. A radial colony of Thiothrix growing in nature. Photomicrograph of living material by Dr. E. J. Ordal.