(From the Biophysical Research Group Utrecht—Delft under the direction of A. J. KLUYVER and J. M. W. MILATZ).

SOME OBSERVATIONS ON THE CULTURE, PHYSIOLOGY AND MORPHOLOGY OF SOME BROWN-RED *RHODOSPIRILLUM*-SPECIES

by

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1. INTRODUCTION.

During an investigation on the genus *Spirillum* carried out by the author (4) 10 years ago in the Laboratory for Microbiology at Delft incidentally also some observations on spirilla were made, which differed from the heterotrophic colourless species by their capacity to carry on a photosynthetical mode of life. Next to various strains which could be identified as *Rhodospirillum rubrum*, also some strains were isolated from mud of a ditch, which clearly distinguished themselves from these, because they did not contain a purple-red, but a definitely brown-red pigment-complex. Moreover these strains were obligately anaerobic. A further investigation showed that the isolated strains represented two different species judged by morphological characteristics. Also some preliminary observations on the physiology of these organisms were made which proved that for the photosynthetical CO_2-reduction substances such as acetate, butyrate, lactate, malate, citrate and pyruvate could function as H-donor.

Though at that time opportunity for a more thorough investigation of these organisms was lacking, and a publication of the obtained data did not seem justified as yet, the results still induced KLUYVER and VAN NIEL (7) to include these spirilla in the natural system of classification of bacteria in a newly created genus *Phaeospirillum*. Unfortunately before long the isolated spirilla were lost, so that no further investigation resulted at that time.

Since then VAN NIEL (14) has published a most valuable study on the culture, physiology and morphology of the non-sulfur purple and brown bacteria, in which he describes the physiological and morphological characteristics of a brown-red, photosynthetically active *Spirillum* isolated by him. Although this organism clearly distinguished itself from *Rhodospirillum rubrum* by its brown-red colour, VAN NIEL's conclusion based on a further study of the physiology of the organism was, that the differences in physiological activity were not sufficient to warrant the placing of this organism

in a separate genus *Phaeospirillum*. In consequence hereof *Phaeospirillum* Kluyver et van Niel was cancelled by VAN NIEL, and the new spirillum was described under the name of *Rhodospirillum fulvum*.

Rhodospirillum fulvum particularly distinguished itself from *Rhodospirillum rubrum* by its brown-red colour. In accordance herewith VAN NIEL found for *Rhodosp. fulvum* an absorption maximum at 530 μ against 553 μ for *Rhodosp. rubrum*. An obvious distinction is moreover that *Rhodosp. fulvum* is a strict anaerobe, whereas *Rhodosp. rubrum* can effect a normal respiration. Fatty acids and the four carbon dicarboxylic acids, besides ethanol, glucose and aspartic acid were found to be suitable substrates for *Rhodosp. fulvum*. There was no development with thiosulfate.

VAN NIEL's investigation was seriously hampered by the great sensitiveness of *Rhodosp. fulvum* towards oxygen. An extensive investigation was not possible, as the four isolated strains soon perished.

The data described in this publication on some brown-red spirilla are the result of an investigation carried out in 1946 in the Physical Laboratory at Utrecht, as part of the program of the Biophysical Research Group Utrecht-Delft.

2. ENRICHMENT AND ISOLATION.

The brown-red spirilla isolated by me 10 years ago originated from an enrichment culture for purple bacteria according to MOLISCH (11), which was inoculated with some mud from a ditch, hay being used as an organic substrate.

Rhodospirillum fulvum was isolated by VAN NIEL from an enrichment culture in a glass stoppered bottle (inoculated with a little mud or surface water), which next to the usual mineral salts as $MgCl_2$, K_2HPO_4 and $(NH_4)_2SO_4$ contained $NaHCO_3$ or Na_2CO_3 (as a supply of CO_2) with the addition of caprylate or pelargonate as the only organic substance. However, VAN NIEL mentions that these enrichment cultures only incidentally led to brown-red spirilla, and he emphasizes that a specific enrichment medium for *Rhodosp. fulvum* is not known as yet.

In my attemps to isolate again brown-red coloured, photosynthetically active spirilla, several times enrichment cultures were incubated in the light cabinet at 25° C. In these experiments the above mentioned basal medium (pH $\pm$ 7.3) was used, after a single organic substance had been added, for which a number of individual members of the groups of simple alcohols, polyalcohols, fatty acids, hydroxyacids and dibasic acids were used. Though in fact in these enrichment cultures incidentally brown-red spirilla developed and could be isolated (among others with lactate, malate, butyrate, ethanol), nevertheless the results were far from satisfactory. A really good enrichment culture could never be obtained, the results being far too erratic, my results being thus in accordance with those of VAN NIEL. Usually *Rhodosp. rubrum* and other members of the

non-sulfur purple or brown rod-shaped bacteria predominate. The exclusive use of the synthetic enrichment media most likely is the reason why VAN NIEL could merely isolate a single brown-red *Rhodospirillum*-species although several are rather common in ditch- and canalwater.

Anyhow during the subsequent investigation it appeared more or less to my surprise that — just as with the heterotrophic *Spirillum* species — a very good and constant enrichment of brown-red spirilla can be secured by means of hay infusion. If namely a glass stoppered bottle filled with water is provided with some hay and the bottle subsequently is inoculated with some mud from a ditch, it is often found that after about one week incubation in the light cabinet at 25° C. the contents of the bottle turn intensely brown-red owing to an abundant development of beautiful spirilla. Among these spirilla mostly two types predominate, namely a very large stout spirillum (under these conditions usually with only $\frac{1}{2}$ to one turn), and a small, slender spirillum. Both types had already been noted by me 10 years ago and subsequently isolated.

The spirilla show a very strong negative aerotaxis. After lifting the stopper of the bottle, the spirilla can be observed to disappear rapidly from the upper layers of the liquid and to aggregate at the bottom of the bottle. This behaviour already suggests the strongly anaerobic nature of these organisms which lateron could be demonstrated on the pure cultures obtained. In view of the anaerobic nature of these spirilla I was somewhat surprised that enrichment cultures of these bacteria could also, and rather often, be obtained from superficially skimmed off canal water.

Another remarkable feature of these enrichment cultures with hay infusion is that not seldom after some weeks tiny green bacteria develop, as a result of which the contents of the bottle turn a dark green. Pure cultures of the bacteria could be obtained via shake-cultures in tap water agar (2 %) provided with 0.05 % K_2HPO_4, 0.05 % $MgSO_4$, 0.1 % NH_4Cl, 0.1 % $Na_2S \cdot 9H_2O$ and 0.1 % $NaHCO_3$, pH $\pm$ 7.3. They proved to be irregularly shaped bacteria and could be identified with *Chlorobium limicola*.

In connection with the isolation of the spirilla here follows an expedient method for the separating of the spirilla from an enrichment culture, in which always a rather high percentage of „contaminating" bacteria occurs. In this method use is made of the capacity of the latter to move very quickly over comparatively large distances. This principle was for the first time applied by me in my study on the heterotrophic spirilla when attempting to isolate the spirilla occurring in liquid media. For that purpose I let the spirilla present in an enrichment culture swim through a „Pasteur-pipette", which was filled with a sterile liquid medium. A number of the very fast moving spirilla very soon leave the non-motile, resp. less motile, bacteria of the enrichment culture far behind, a fact which can be followed fairly easily under the microscope. By cutting the pipette in the right place it thus was

possible to separate a number of spirilla from the ,,contaminating" bacteria.

The same method has later been applied by MYERS (12) for the mechanical separation of spirilla from crude cultures. The method, however, has now been further simplified by me in the following way.

A tiny drop of the enrichment culture is brought on a glass slide, after which a drop of sterile tapwater or of a nutrient liquid (which in the case of the brown-red spirilla should be boiled beforehand in view of the strongly negative aerotaxis!) is placed on the slide very close to the drop of the enrichment culture. Both drops are now carefully put into contact with each other, and in that way are united into one big, preferably somewhat protracted drop. Through the microscope it can now be observed that the very motile spirilla rapidly disperse into the sterile part of the drop. After some minutes already quite a lot of the spirilla have reached the other extremity of the ,,sterile" part of the drop. By means of a finely drawn-out micropipette it is possible to remove under the microscope a little of the ,,sterile" liquid containing a large quantity of spirilla.

Thus very quickly and without much difficulty a particularly effective separation of the spirilla from the non-motile, resp. less motile, organisms in the enrichment culture is secured. Not seldom in using this procedure 80—90 % of the developing colonies after inoculation in a nutrient agar proved to be colonies of the desired spirillum. This, of course, considerably simplifies the necessary microscopical examination of the colonies and the isolation of the spirilla. Naturally the method is not only valuable for the mechanical enrichment of spirilla but can be used whereever the separation of motile from less motile, resp. non-motile, microorganisms is required.

For the isolation of the brown-red spirilla shake-cultures in culture tubes were made [1]). As agar medium peptonagar (Pepton Poulenc) proved to be satisfactory. The pH should be neutral to slightly alkaline. After a lapse of about one week one observes in the agar, between cultures of the much faster developing ,,contaminating" bacteria, small brown-red coloured colonies which at microscopical examination turn out to consist of spirilla.

To transfer these colonies for further purification, for convenience sake [2]), a simple micromanipulator was used by means

[1]) VAN NIEL recommended as a practical measure for isolation purposes (shake-cultures) the use of soft glass rather than of Pyrex tubes, because when the agar has to be removed the former tubes can be easily cut at the bottom. However, the agar column can also rather easily be removed from the glass tube without the need of cutting the tube. Hereto a vigorous air current is blown through a Pasteur-pipette into the tube between the agar column and the wall of the tube. If the air current is strong enough, the air penetrates between the agar wall to the bottom of the tube, finally causing the whole agar column to be pressed out of the tube.

[2]) A great advantage of the use of the micromanipulator is the con-

of which the spirilla were sucked in finely drawn- out micropipettes. For that purpose 1—2 mm thick slices of the agar column were cut with a razor blade. In case there are many colonies of other bacteria in the slices there is a chance that during the cutting of the agar the flat of the cut will become contaminated with these bacteria, so that on sucking the spirilla in the micropipette numerous ,,contaminating" bacteria will be carried along with the spirilla. However, this can for the greater part be prevented in a simple way by carefully washing the agar slice lying on the slide for some time under a thin jet of tapwater. In this way the flat of the cut can be cleaned to a large extent of loose bacteria. The excess tapwater can then be sucked off with filter-paper, and subsequently the slice of agar superficially dried at 30° C. Thus it can be effected that already after one transfer nearly all ,,contaminating" bacteria are eliminated.

When the agar column does not contain many colonies it is still better to break the agar column cautiously by bending it. Then with a razor blade a slice of the agar can be cut, taking care in doing so that the break remains untouched.

When making transfers of the colonies it is necessary to take into account the strictly anaerobic nature of the brown-red spirilla. Without taking special precautions disappointments are bound to occur because the transferred spirilla will not develop in the fresh agar. These precautions do not merely consist in the boiling of the agar before the transfer in order to remove the oxygen, but als in adding an amount of about 0.01 % $Na_2S \cdot 9H_2O$, and in covering the agar colum with a thin layer of sterile paraffin of low melting point. For safety's sake I also added 0.1 % $NaHCO_3$ to the agar as a CO_2 source though this is not strictly necessary, because the brown-red spirilla can also grow in pepton agar without special addition of CO_2. Lastly the oxygen in the air column above the agar was removed by bringing in the tube some cotton-wool soaked in a mixture of pyrogallic acid and potassium hydroxide and by subsequently closing the tube with a rubber plug. Even with these precautions it is advisable, when making transfers, to blow the spirilla out of the micropipette into the lower part of the liquid agar medium, because the spirilla often refuse to grow in the uppermost agar layer of about 1 cm.

In the course of the investigation about 20 strains of brown-red spirilla were isolated in the described way. The pure cultures were kept at room temperature in day-light as stable cultures in pepton agar. The oxygen was removed from the culture tubes in the above mentioned way. Addition of Na_2S and Na_2CO_3 is not necessary, but covering of the agar with paraffin remains desirable,

siderable gain of time obtained. In that way it is often possible to make transfers already after 1—2 weeks. In order to obtain colonies sufficiently big for transferring by hand, one has to wait much longer. Moreover, the use of the micromanipulator offers better chances of avoiding contaminating bacteria.

whilst the agar should be boiled well before transferring. When making transfers it is certainly to be recommended for a successful start of the culture to inoculate heavily, which can be done better by means of a Pasteur-pipette than with a platinum needle.

3. CLASSIFICATION AND IDENTIFICATION OF THE ISOLATED SPIRILLA.

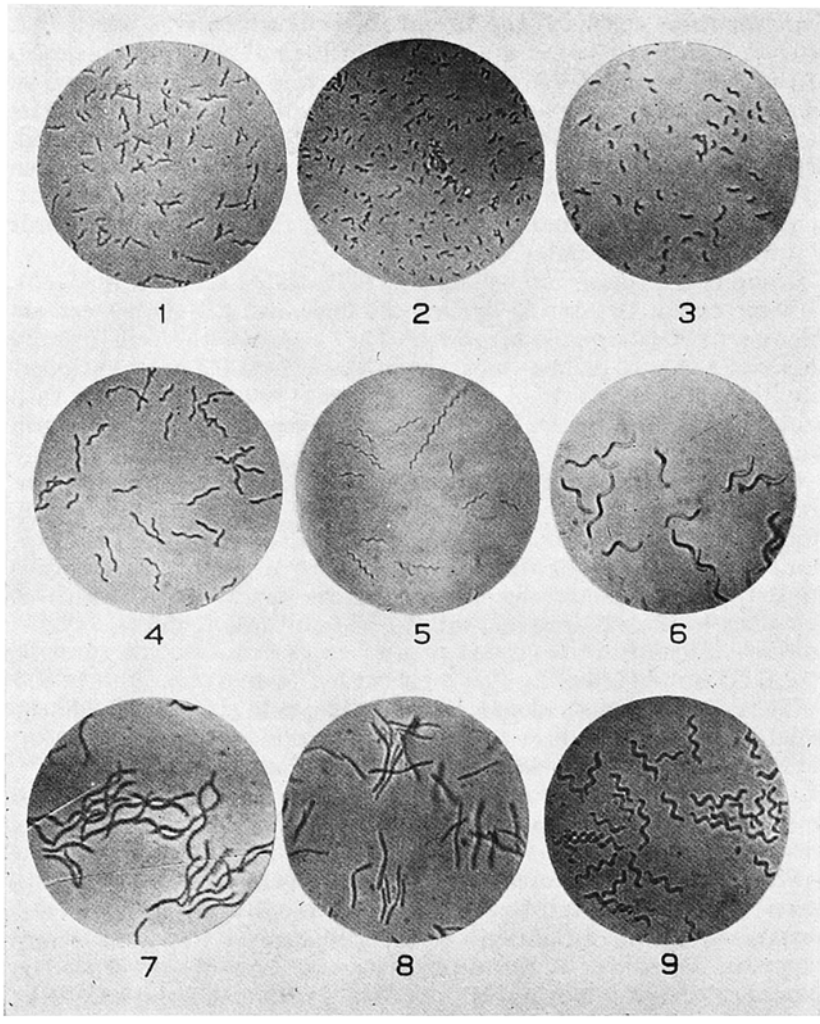
a. Introductory. In accordance with the experiences obtained in my study of the heterotrophic spirilla, VAN NIEL emphatically points out the great morphological variation which *Rhodosp. rubrum* and *Rhodosp. fulvum* show under various environmental conditions. Hereby a good description and identification of these organisms was seriously hampered. Hence VAN NIEL's statement that an adequate description of a species which shows such a pronounced morphological variability must include the characteristics of the organisms as it develops under a variety of environmental conditions (e.g. the reaction of the medium, the nature and the concentration of the nutrient materials present etc.). The judgment of the conditions which should be regarded as representing a more or less normal and significant state is hereby, at least partly, a matter of personal appreciation.

The same difficulty had to be faced when trying to give a morphological characterisation of the brown-red spirilla isolated by me. Although the isolated strains could be separated into three, morphologically clearly distinct groups, the strains comprised in these groups to a large extent exhibit the phenomenon of morphological variation under various environmental conditions (compare fig. 1—9). Even variations in environmental conditions so slight that at the present time it remains impossible to evaluate them, cause one and the same organism to manifest itself either as a nearly straight rod-like bacterium with only faintly developed turns, or in the form of beautifully spiral-shaped cells. Between these extremes various intermediary forms can occur. It is almost impossible to include these divergent forms in a satisfactory morphological characterisation.

With regard to the foregoing remarks it must immediately be emphasized, however, that the spirilla from prolific cultures — with practically all the cells strongly motile — always exhibit normal spiral-shaped cells. The very flatly wound, almost straight cells are only found in cultures which obviously do not represent optimal conditions. An indication hereof is the fact that the cells mostly are but slowly motile, whereas in many cells there is a tendency to retarded division and thus to grow out into very long cells.

However divergent the shape of the turns may be, generally the thickness of the cells does not vary much. In my opinion therefore this quality is one of the most important criteria for the morphological characterisation.

In the description of the morphological properties following below I have attempted to give a characterisation of the cell form of the spirilla in actively proliferating cultures.



Figs. 1—2. *Rhodospirillum fulvum* (Group I). × 460. Cultures of strain 1 in basal medium with lactate and asparagin respectively.
Figs. 3—5. *Rhodospirillum Molischianum* (Group II). Figs. 3 and 4: × 460; Fig. 5: × 345. Cultures of strain 5 in basal medium with ethanol and citrate respectively, and in 0.2 % pepton 0.5 % Ca-lactate.
Figs. 6—9. *Rhodospirillum photometricum* (Group III). Fig. 6, 7, 8: × 460; Fig. 9: × 345. Cultures of strain 17 in hay-infusion, pepton water, 1 % pepton, 0.5 % Ca-lactate and 0.2 % pepton, 0.5 % Ca-lactate respectively.

b. Morphological properties of the isolated strains.

As mentioned before, the isolated strains fell apart in three clearly distinct groups.

Group I. To this group belong 2 strains [1]) (fig. 1—2). Characteristic for these spirilla is the size of the cells which is so small that often it is difficult to get a good idea of their shape. The thickness of the cells is 0.3—0.4 μ, whilst the length is mostly only 2—3 μ. In media to which certain salts of organic acids, for instance malate, have been added, sometimes individual cells can be encountered, which are 4—5 μ long. In case salts of organic acids have been added the spirals often manifest themselves more distinctly, but, in view of the small dimensions of the cells, the width of the spirals is difficult to appreciate.

Group II. To this group belong 8 of the isolated strains (fig. 3—5). To some extent the spirilla in their cell form and size show a certain likeness to *Rhodospirillum rubrum*. The length of the cells usually amounts to 5—10 μ. The thickness of the cells is 0.7—0.9 μ. Generally the cells shows 1—2 complete turns, the length of a turn being 4—6 μ, and the width 1.3—2 μ. As reserve substance volutin is found.

Group III. In this group the remaining 11 strains could be included (fig. 6—9). Characteristic for these spirilla are the large, stout cells. The length of the cells mostly amounts to 14—30 μ, while the thickness of the cells is 1.2—1.5 μ. Generally the cells have 1—2 complete turns. Length of the turn 7—9 μ, width of the turn 4—6 μ. As reserve substance volutin is found.

c. Colour of the isolated strains.

The colour of the colonies and of the stab cultures of all the isolated strains was brown-red, the colour of Group I, however, showing a somewhat redder tinge than that of the other strains [2]). The colour of liquid cultures could often be designated as brown-red to brown-orange. Formation of water soluble, diffusable pigments was not observed.

The absorption spectrum of thin suspensions of living cells in water was determined by the method usually applied for this purpose in the investigations of the Biophysical Research Group (compare WASSINK, VERMEULEN, REMAN and KATZ (17)). The apparatus mainly consists of a double monochromator according to VAN CITTERT and an alternating current amplifier according to MILATZ (9, 10). For comparison the absorption spectrum of a suspension of living cells of *Rhodospirillum rubrum* is also included in fig. 10.

The figure immediately strikes by the great conformity in the absorption spectra of the three groups of brown-red spirilla. Meanwhile there are also differences. Characteristic for Group III is for

[1]) Unfortunately both strains died during a severe frost period when the laboratory was not heated.

[2]) It is striking that there exist different species of brown-red spirilla, whereas up to now *Rhodospirillum*-species with the same pigment complex as *Rhodosp. rubrum*, but differing from this species in other respects have not as yet been isolated with certainty.

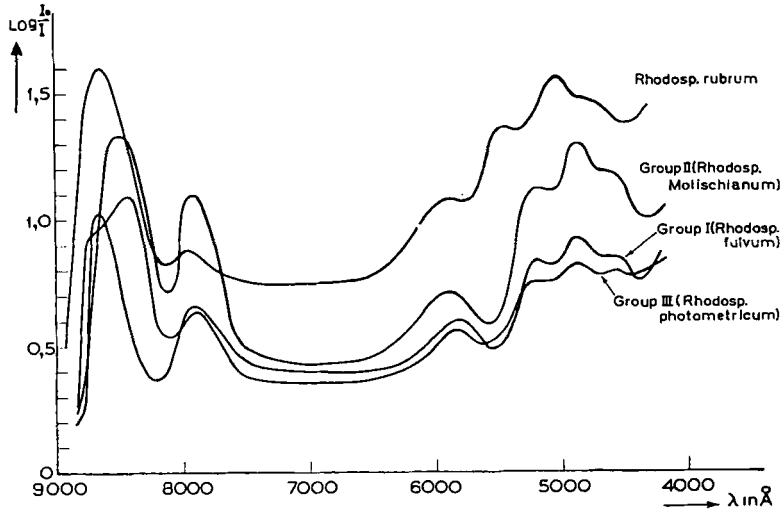


Fig. 10. Absorption spectra of suspensions of living cells of four *Rhodospirillum*-species.

instance the typical slanting maximum (of bacteriochlorophyll) at ± 8500 Å. This can be explained by the presence of two closely adjoining maxima, which in view of the theoretical considerations of WASSINK, KATZ and DORRESTEIN (16) and of FRENCH (3) must be connected with the properties of the proteins, to which the bacteriochlorophyll is bound.

On comparing the spectra with the infrared absorption spectra of various purple sulfur bacteria given by WASSINK *et al.* (16) it is of interest to note that such a flat maximum of the bacteriochlorophyll in the infrared part of the spectrum is encountered in morphologically very divergent groups of organisms. The absorption spectrum of the brown-red spirilla of Group III for instance is in this respect entirely comparable with the absorption spectrum of *Phaeomonas* strain 4 (compare WASSINK *et al.*).

The most typical difference between the absorption spectra of the brown-red spirilla and that of *Rhodosp. rubrum* is the absence of the maximum at 5500 Å in the absorption spectra of the brown-red spirilla. For the rest some more differences in the position of the maxima between the absorption spectra of the brown-red spirilla and *Rhodosp. rubrum* appear at the shorter wave lengths. Moreover, one is struck by the phenomenon, that the maximum at 7900 Å of the bacteriochlorophyll, which manifests itself only faintly with *Rhodosp. rubrum*, is very conspicuous in the absorption spectra of the brown-red spirilla.

From the investigation of MANTEN (8) it is evident, that the maximum at 5500 Å in the absorption spectrum of *Rhodosp. rubrum* is due to the occurrence of the carotenoid spirilloxanthin (rhodoviolascein). From the absorption spectra of the brown-red

spirilla thus the conclusion can be drawn that this carotenoid is absent in these organisms. The results of a chromatographical analysis of the pigment complex of one of the strains of Group III were in perfect agreement with this conclusion. By this chromatographical analysis next to bacteriochlorophyll the occurrence of 5 different carotenoids could be established. A detailed report of this investigation will be published elsewhere.

d. Physiological properties of the isolated strains.

For their development the brown-red spirilla are bound to a neutral to slightly alkaline reaction of the medium. The influence of the temperature has not been investigated in detail, but growth of the spirilla was good between 25 and 35° C.

All isolated strains were strictly anaerobic. Further details about the great sensitiveness of these organisms to oxygen have been given in the discussion of the isolation of the spirilla. Adaptation to oxygen has not been observed. In this respect the brown-red spirilla distinguish themselves markedly from *Rhodosp. rubrum* for their growth is strictly dependent on photosynthetical activity, whereas *Rhodosp. rubrum* can also grow in the dark by means of a respiration process.

Gelatin is not liquefied by any of the isolated strains.

e. Biochemical properties of the isolated strains.

In order to ascertain which organic substances could function as a substrate (H-donor) for the development of the spirilla, the isolated strains were inoculated in liquid media to which besides the usual inorganic nutrients and 0.1 % $NaHCO_3$ 1 % of certain organic substances was added.

No differences in this respect could be established between the three groups of strains. All the strains developed more or less satisfactorily with substances such as: acetate, butyrate, malate, lactate, citrate, succinate, ethanol and asparagin. With glucose no growth could be observed of the strains belonging to group II or III [1]).

Thiosulfate nor Na_2S were utilized. The isolated brown-red spirilla thus belong to the typical *Athiorhodaceae*.

f. Identification of the isolated strains.

Besides the well-known and frequently investigated *Rhodosp. fulvum* described by VAN NIEL a few other *Rhodospirillum*-species have been recorded in the literature. So MOLISCH (11) in his well-known monograph on the purple bacteria has described two *Rhodospirillum*-species, nam ly *Rhodosp. photometricum* and *Rhodosp.*

[1]) The strains of group I have not been examined in this respect, because they had already perished at that time.

giganteum, from which only the first species has been studied in pure culture [1]).

Both species have been declared by VAN NIEL to be synonymous with *Rhodosp. rubrum* because in his opinion the morphological properties of the species described by MOLISCH, in view of the great morphological variation of these organisms do not justify a differentiation from *Rhodosp. rubrum*. However, in my opinion this cannot be accepted without further argumentation as MOLISCH explicitly mentions that he possessed an authentic culture of *Rhodosp. rubrum*, and that it thus should be accepted, that MOLISCH for good reasons considered *Rhodosp. rubrum* and *Rhodosp. photometricum* to be different species. Though in fact *Rhodosp. rubrum* shows a great morphological variation, it is still an exception that the individual cells attain a thickness of more than 1 μ (thickness of the cells generally 0.7 μ), whereas for *Rhodosp. photometricum* the thickness of the cells is stated by MOLISCH as 1.4 μ.

In this respect it is also important to mention that VAN NIEL's own comment on the spectroscopic investigation of MOLISCH was, that MOLISCH has actually investigated spirilla with a pigment complex different from that of *Rhodosp. rubrum*! It seems very probable that *Rhodosp. rubrum* and *Rhodosp. photometricum* are two different organisms.

Another question is whether it is justified to maintain a separate species *Rhodosp. giganteum* apart from *Rhodosp. photometricum*. MOLISCH has described the species *Rhodosp. giganteum* on account of the dimensions of this organism in an enrichment culture. A pure culture of the spirillum was not obtained. The differentiation in dimensions between *Rhodosp. giganteum* and *Rhodosp. photometricum*, however, is only slight, and might entirely be attributed to morphological variation. I too have several times observed, in enrichment cultures, a luxurious development of brown-red spirilla having a somewhat more slender appearance than the spirilla belonging to group III generally show in a crude culture. Nevertheless on isolation it always turned out that the developing colonies only consisted of spirilla representing the normal type of group III, so that the primarily observed differences may well be due to morphological variation. This may explain why MOLISCH could not secure pure cultures of *Rhodosp. giganteum*. In my opinion therefore, *Rhodosp. giganteum* should be considered as synonymous with *Rhodosp. photometricum*.

Next to the above mentioned species HAMA (5, 6) has described *Rhodosp. longum* and *Rhodosp. gracile*, though both only on the basis of the shape of the cells in crude cultures. These species with regard to their morphological properties take a somewhat inter-

[1]) NAKAMURA (13) in a publication has claimed the isolation of *Rhodosp. giganteum*. However, NAKAMURA's data on the morphological properties of the isolated spirillum are entirely inadequate for this conclusion. As the spirillum isolated by NAKAMURA could utilize Na_2S and thiosulfate as only H-donor, it belongs to the *Thiorhodaceae*.

mediate position between *Rhodosp. rubrum* and *Rhodosp. photometricum*. Since further data about the properties of these species are lacking, the available data on the morphological properties, in view of the great morphological variability, do not justify a differentiation of these species from *Rhodosp. rubrum* resp. *Rhodosp. photometricum*. In accordance with VAN NIEL who considers both species as synonymous with *Rhodosp. rubrum*, in my opinion the species described by HAMA cannot be maintained as separate species.

After this critical review of the *Rhodospirillum*-species recorded in the literature, it remained to ascertain whether the strains isolated by me could be identified with already described species.

Group I. The fact that the dimensions of the cells of both strains isolated by me are identical with those of *Rhodosp. fulvum* has removed any doubt for me that both these strains can be identified with *Rhodosp. fulvum* van Niel.

Group II. With regard to the dimensions of the cells, the strains belonging to group II show a rather close agreement to *Rhodosp. rubrum*, but they differ from these species by the nature of their pigments and by their different behaviour towards oxygen. Obviously here we are dealing with a new, not yet described species. With regard to the fact that MOLISCH was the first to observe brown-red spirilla (see later), the name of *Rhodospirillum Molischianum* is proposed for the organism.

For the sake of completeness the diagnosis of *Rhodospirillum Molischianum* nov. spec. is given here:

Moderately large, very motile, spiral shaped cells. Length of the cells 5—10 μ, thickness of the cells 0.7—0.9 μ. Cells mostly with 1—2 complete turns. Length of the turn 4—6 μ, width of the turn 1.3—2 μ. As reserve substance volutin is found. Obligately photoheterotrophic. As substrate various organic substances, such as acetate, butyrate, malate, lactate, citrate, succinate, ethanol, asparagin can be utilized. Glucose and glycerol are not utilized, no more than Na_2S and thiosulfate. Gelatin is not liquefied. Colour of the cells brown-red. Besides bacteriochlorophyll various carotenoids are found as pigments, but not spirilloxanthin.

Group III. The spirilla belonging to this group are particularly characterized by the stoutness of their cells, the dimension of which are on the whole in good agreement with those given by MOLISCH for *Rhodosp. photometricum*. The photographs of this organism made by MOLISCH on the whole are in good agreement with the appearance of the strains isolated by me.

It is also important to observe that both MOLISCH and I successfully used the same enrichment medium, namely hay-infusion. It is highly probable, that MOLISCH in doing so has regularly obtained cultures of brown-red spirilla. In accordance with this MOLISCH writes about the enrichment cultures of *Rhodosp. giganteum*, that „das Wasser bis hinauf rotbraun gefärbt erscheint". VAN NIEL also gives his opinion that in view of the spectroscopic investigations of MOLISCH on the pigments of *Rhodospirillum*-species one must

conclude that MOLISCH has investigated spirilla with a brown-red pigment complex.

On the basis of the preceding considerations I conclude that it is justified to identify the strains belonging to group III with *Rhodospirillum photometricum*.

The following diagnosis of *Rhodospirillum photometricum* Molisch can be given:

Large, stout, very motile spiral shaped cells. Length of the cells 14—30 μ, thickness of the cells 1.2—1.5 μ. Cells mostly with 1—2 complete turns. Length of the turn 7—9.5 μ, width of the turns 4—6 μ. Volutin is found as reserve substance. Obligately photoheterotrophic. As substrate various organic substances such as acetate, butyrate, malate, lactate, citrate, succinate, ethanol, asparagin can be utilized. Not utilized are glucose and glycerol, no more than Na_2S and thiosulfate. Gelatin is not liquefied. Colour of the cells brown-red. Besides bacteriochlorophyll various carotenoids occur as pigments, but not spirilloxanthin.

Summary.

From ditch- and canalwater 21 strains of brown-red, photosynthetically active spirilla were isolated. These spirilla distinguished themselves from *Rhodospirillum rubrum* by the composition of their pigment complex and by their behaviour towards oxygen (strictly anaerobic). The strains, judged by morphological characteristics, represented three different species. Two of the species could be identified with *Rhodospirillum fulvum* van Niel resp. *Rhodospirillum photometricum* Molisch. The third species was found to be a new one, for which the name *Rhodospirillum Molischianum* was proposed.

Regarding the isolation, culture, morphological and physiological characteristics of these organisms detailed data were given. Various organic substances can function as H-donors for these spirilla. The absorption spectra of living cells of the three species show a striking conformity, but there is a distinct difference between the absorption spectra of the brown-red *Rhodospirillum*-species and the absorption spectrum of *Rhodospirillum rubrum*. The results of a chromatographical analysis of the pigment complex of one of the strains of *Rhodospirillum photometricum* make it probable that this difference is due to the absence of the carotenoid spirilloxanthin in the brown-red spirilla.

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