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$$a_{2k+1} = 0 \qquad a_{2k} = (-1)^k \frac{H_{2k}(1)}{2^{2k}}$$

for then

$$f(y) = \frac{1}{\pi} \int_0^\infty \cos yu \sum_0^\infty \frac{H_{2k}(1)}{(2k)!} \left(\frac{u}{2}\right)^{2k} du$$

or, according to the expansion II

$$f(y) = \frac{1}{2\pi} \int_0^\infty \cos y u \, e^{-\frac{u^2}{4}} (e^u + e^{-u}) \, du,$$

which reduced by *b* Art. 6, gives

$$f(y) = \frac{e}{\sqrt{\pi}} e^{-y^2} \cos 2y \, .$$

Microbiology. — *"On the nitrate ferment and the formation of physiological species".* By Prof. Dr. M. W. BEIJERINCK.

(Communicated in the meeting of March 28, 1913).

It is a well-known fact that in soil as well as in liquids containing a great many individuals of the nitrate ferment, large amounts of organic substances may be present without preventing nitratation, which is the oxidation of nitrites to nitrates by that ferment.

On the other hand it is certain, that when only few germs of the ferment are present, so that they must first grow and multiply in order to exert a perceptible influence, extremely small quantities of organic substance are already sufficient to make the experiments fail altogether, the nitrite then remaining unchanged in the culture media.

It is generally supposed, that this latter circumstance must be explained by accepting that the nitrate ferment can only then grow and increase, when soluble organic substances are nearly or wholly absent.

My own experiments, however, have led me to quite another result, namely that the nitrate ferment very easily grows and increases in presence of the most various organic substances. But in this case, that is, *when growing at the expense of organic food*, it soon wholly loses the power of oxidising nitrites to nitrates and then changes into an apparently common saprophytic bacterium.

This change may be called the formation of a physiological species, and the two conditions of the ferment thus resulting, respectively the *oligotrophic* and the *polytrophic* form.

Furthermore it is proved that the usual laboratory experiments cannot give back to the polytrophic form, when it is kept in absence of soluble organic matter and cultivated in a dilute nitrite solution, the power of oxidising nitrites, not even in the course of 10 years.

Consequently the process of nitratation in pasture ground must take place as follows.

When the soil contains a great deal of organic matter this need not be exclusively oxidised and destroyed by other species of bacteria in order to make the action of the nitrate germs possible, but it may also be done in part of the germs themselves. It is true that they get lost thereby as they pass into the polytrophic form, but in the soil always places must be present without any considerable quantity of organic substance, where unchanged oligotrophic germs occur. These, after the destruction of the organic matter in their environment, can multiply and again provide the soil with a new nitratating flora.

It is very difficult to obtain pure cultures of the nitrate ferment in the nitratating or oligotrophic condition. The best way is as follows. First a crude nitratation is produced by bringing pasture soil into a liquid of the composition: tapwater 100, sodiumnitrite 0,05 to 0,1, bipotassiumfosfate 0,01, and cultivating at 30° C.

After about one or two weeks the nitrate ferment of the infection material has strongly increased and all the nitrite may be converted into nitrate.

A little of this nitratation, diluted with much water, is now sown on the surface of a plate of the composition: tapwater 100, carefully extracted[1]) agar 2, sodiumnitrite 0,05, potassiumfosfate 0,01, and again cultivated at 30° C. As the nitrate ferment and the other microbes accumulating in the crude nitratations, do not attack the agar, the rate of soluble organic food present is very low. The nitrate ferment can grow upon such a plate without losing its faculty of nitratation and forms very minute colonies of about $^1/_2$ to 1 mill. in diameter, which, being very transparent and glassy little discs, are hardly visible. With a greater percentage of water in the agar they are dendritically branched, with a smaller percentage they remain unchanged circular, or become somewhat crenate. In such a pure culture the distance between the colonies must be so great, that they do not touch one another and can be separately examined. In consequence their number on the plates must be relatively small, the counteraction of the still remaining soluble organic substances great, and the nitratative power

[1]) For the extraction the agar is left many days in distilled water which is now and then renewed.

feeble. Hence the experiment takes much time, two or three weeks or longer.

To obtain pure cultures on silica plates is much more troublesome than on carefully extracted agar, although the nitratation takes place very easily on that medium.

Some other species of bacteria, eventually occurring in the crude *nitratations, may produce colonies* on the agar much resembling those of the nitrate ferment. Those species which in the crude nitratations do not multiply at all or only very little, are to be recognised on the plates by their relative rarity. But there exists a species, the denitrifying, spore-forming *Bacillus nitroxus* [1]), which can increase in *the nitratating fluid and on the plates with the same intensity as* the nitrate ferment itself and whose separation from the latter gives rise to difficulties. But here the formation of a new physiological species comes to our assistance, in as much as the nitrate ferment, on broth- or peptone agar, produces very characteristic although no more nitratating colonies. They are white-coloured, extensive and *thin; at first dry and flat, they* later become thicker, slimy and moist, and are easily distinguished from the small, semi-spherical, moist *Nitroxus* colonies.

On the nitratating plates may further be found the colonies of *Bacillus oligocarbophilus*, which are directly recognised by their white colour and paperlike appearance, and to which I shall return later. Moreover a most characteristic species resembling *Actinomyces*, but in fact nearly allied to *B. oligocarbophilus*. So, in all four species which should be considered as characteristic for the crude nitratations, because, after repeated transplantations they never dissappear, whilst the numerous other species eventually obtained, are but *accidental inhabitants and at continued transferring* to fresh media may be quite expelled.

When the pure cultures of the nitrate ferment are kept in continual contact with the above nitrite solutions, or on the nitrite agar plates poor in soluble organic food, the faculty of oxidising nitrites to nitrates remains unchanged, probably for an unlimited length of time, that is, the ferment preserves its oligotrophic or oligophagic condition. Microscopically it makes the impression of a small *Micrococcus* but in reality consists of very short rodlets of $0,2 \times 0,1\ \mu$, *which in nitratating condition always seem non-motile.*

If the ferment is now transferred to solid media or to culture liquids richer in organic food, as for instance broth agar or agar

1) To compare: Bildung und Verbrauch von Stickoxydul durch Bakterien. Centrabl. für Bakteriologie 2te Abt. Bd. 25, S. 30, 1910.

dissolved in water with $^1/_{20}$ % peptone or more, it grows, as said, vigorously and produces colonies of the above nature, *in which it is always possible to detect some few motile bacteria.* The rodlets now become somewhat longer and thicker than in the nitratating state but for the greater part they remain very short; the ferment has now changed into the polytrophic form.

In broth the same change takes place already on the second or third day, at 30°., fairly rich cultures being obtained, whereby the broth becomes distinctly turbid and is sometimes covered with a thin film, perfectly resembling that of *B. oligocarbophilus.* In the liquid thin rodlets and threads are found, many of which are moving. They never ramify and their motility shows that they do not belong to the family of the Actinomycetaceae, although their way of growing might suggest it. Accordingly the statement in the manuals, that the nitrate ferment may be recognised by its not growing and increasing in pure culture in broth, is quite erroneous, only nitratation is excluded.

On broth-gelatin plates at room temperature the growth is at first rather slow but very characteristic and finally fairly strong, where by the gelatin quite liquefies and much ammonium carbonate is produced.

On pure gelatin, dissolved in distilled water, with nutrient salts, hardly any development is visible, the nitratative power is nevertheless rapidly destroyed.

The quantity of dissolved matter required to destroy this faculty, is extremely small. Media with $^1/_{20}$ % of substances such as glucose, mannite, asparagin, peptone, tyrosin, natriumacetate, or calciumacetate, cause a vigorous growth and total loss of the nitratative function. With a much smaller amount of soluble organic substance in the medium, for example that of common non-extracted agar, the nitrate ferment is able to assimilate that slight quantity without losing the faculty of nitratation. But under these circumstances weeks or months are required for the oxidation of the nitrite, and many experiments fail altogether. Old dung, such as is found in dung-heaps, does not destroy the faculty; vegetable juices, pressed out from stems and roots of plants, convert the nitrate ferment into the polytrophic, non-nitratative form, which conversion must, under certain conditions, also take place in the soil.

Humates in the culture liquids or plates, even in rather great quantities, are not assimilated and cause no change in the nitratation.

Addition of paraffinoil slackens the nitratation a little, but does not at all prevent it.

The very striking fact, that the nitrate ferment acts best when organic substances are as far as possible kept out of the cultures, has suggested the supposition that this microbe could feed by chemosynthesis, whereby the energy, produced by the oxidation of the nitrites should serve for the reduction of atmospheric carbonic acid.

For this hypothesis I have not, however, been able to find a single proof.

When the pure nitrate ferment is cultivated in a liquid this remains quite clear; only with the microscope many bacteria can be detected, especially on the glass wall. A particle of cotton wool fallen from the air into the cultures, represents a quantity of organic matter equalling some millions of nitrate bacteria.

On silica plates soaked with solutions of 0,1 °/₀ to 0,05 °/₀ sodium nitrite and 1,01 °/₀ bipotassium fosfate, the nitrate ferment always forms small but very active colonies only visible when magnified *and the smaller as the organic matter is better removed from the plates.* So there is also here ground to suppose that for the carbon requirement of these extremely small colonies, always a sufficient amount of organic substance is present in the impurities of the plates.

But the strongest argument against the existence of chemosynthesis with regard to the nitrate ferment, is the following circumstance.

The crude cultures are always covered in the laboratory with a thin, floating film, consisting of the above mentioned highly remarkable bacterium, described by me in 1903 under the name of *Bacillus oligocarbophilus*.[1]) When the nitratation experiments are effected in a hothouse this film also appears but later and then it always remains much thinner. When such nitratations are sown out on agar- or silica-plates, *Bacillus oligocarbophilus* likewise forms colonies, which at first sight reveal their relation to the nitrate ferment, but they grow out considerably larger and finally have the appearance of snow-white, dry, flat plates of one or more millimeters in diameter. As *B. oligocarbophilus* is not able to oxidise nitrites, and hence, under the said circumstances does certainly not possess the power of reducing carbonic acid by chemosynthesis, there must evidently be in the environment a sufficient amount of fixed organic carbon to provide the carbon requirement of this species. As the nitrate ferment not only lives in the same fluids as *B. oligocarbophilus*, but by the nature of the colonies resembles it very much, and moreover quite corresponds with it as regards its microscopic appearance, its motility

[1]) Farblose Bakterien deren Kohlenstoff aus der atmosferischen Luft herrührt. Centralbl. für Bakteriologie; 2e Abt. Bd. 10. S. 38. 1903.

and its conditions for nutrition in the polyphagous state, it is quite sure that these two bacteria are nearly allied. Consequently we must conclude that the nitrate ferment can feed on the same organic substances, which *B. oligocarbophilus* finds at its disposal, as well in the liquid as in the extracted agar and the silica plates. That those substances are at least partly provided by the atmosphere of the laboratory, I have pointed out in the above mentioned paper.

The nature of these substances is not yet stated, but it is very probable that volatile products, given off by other bacterial cultures occur among them.

In this relation I call to mind the experiment mentioned above with paraffin oil, whose presence does not stop the nitratation. Perhaps the nitrate ferment can feed on it, or on allied substances, whose occurrence in the soil or the atmosphere seems not excluded.

From the foregoing must be concluded, that chemosynthesis for the nitrate ferment is unproved and that, as far as can be judged at present, it is in this case a quite superfluous hypothesis.

Summarising we find, that the nitrate ferment represents a definite state of a greater unity, a physiological species, which may be kept constant in the nearly pure anorganic nitrite solutions, but which, at better nutrition with organic substances, passes into an other state of that unity, another physiological species much more constant.

If the former, that is the nitratating state of the ferment, is called *Nitribacillus oligotrophus,* the latter, non nitratating condition, may be named *Nitribacillus polytrophus.* The conversion of the former into the latter, that is in the direction

N. oligotrophus → *N. polytrophus*

easily takes place; the passage in opposite direction: *N. polytrophus* → *N. oligotrophus,* cannot be effected by the usual laboratory experiments.

Although the nutrition of *Nitribacillus oligotrophus* requires an almost total absence of organic food, there is no cause to ascribe to this ferment the faculty of chemosynthesis.

The question, where the here described case of the formation of a physiological species must be placed in the system of biology, is to be answered as follows.

It cannot be an example of mutation, such as I have amply described for a number of microbes, as the more or less constant products of the mutation process arise at the side of the stock, and continue to exist with it under the most different conditions.

But it is a new case of *hereditary modification;* in fact not much differing from the loss of virulence of many pathogenic bacteria, only much more evident as to the outward characteristics. Comparable to, but not identic, with the pleomorphy of many Fungi; — comparable, too, to the differentiating process in the ontogenetic development of the higher plants and animals, the result of which we observe in the various cell-forms of one and the same individual. By artificial nutrition, and independently of their relation with the other cells, some of these cells can multiply without change of properties, hence, also without returning to the state of the mother-cell or the embryonal cell from which they sprung. The increase of connective tissue and of the muscle cells of the embryonal heart, cultivated in bloodplasm, are good examples.

Finally I wish to remark that the physiological formation of species is not an isolated case for the nitrate ferment, but that it same takes place in the life history of many microbes of soil and water. To these other species, showing the same disposition, belongs *B. oligocarbophilus*, which is, as said, nearly allied to the nitrate ferment itself and on some media cannot even be distinguished from it.

By the isolation of this bacterium on nitrite- or nitrate agar without organic food, the floating films of crude nitratations, colonies are obtained, which by their white, dry surface are perfectly like the films of the culture liquids, and which, when repeatedly transferred on the same medium, without other organic food, can preserve the film-character unchanged in the course of years. But if these cultures are transferred to broth- or peptone agar, their characteristic appearance gets lost, wet and glittering colonies arise, semi-spherical, not extending sideways and seemingly belonging to quite another species. When multiplying they, hereditarily transmit their newly acquired properties, *also when again transplanted on media without organic food.*

The thus obtained polytrophic form and the oligotrophic mother-form, make a couple, quite comparable to the two conditions of the nitrate ferment.

For a long time I considered the polytrophic form of *B. oligocarbophilus* as a wholly different species, always mixed with the primitive stock as an impurity. Erroneously I thought, that the isolation could only be effected by means of a better nutrition, the oligotrophic form thereby dying off. So, I had fallen into the same error as my predecessors concerning the nitrate ferment, but the recognition of the physiological formation of species now brings the required light.

(May 29, 1914.)