Microbiology. — *"Mutation in Penicillium glaucum and Aspergillus niger under the action of known factors."* By H. J. WATERMAN. (Communicated by Prof. M. W. BEIJERINCK).

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A. Penicillium glaucum.

In solutions of *p*- and *m*-oxybenzoic acid a spontaneous growth of mould had developed at the air. From this material, which floated on the liquid, a pure culture of *Penicillium glaucum* was obtained by isolation on malt agar, which culture was used in the biochemical investigations described by Prof. BÖESEKEN and WATERMAN.[1]) It looked quite normally green and had the peculiar "mould smell". The culture was transferred some times in the course of a year; mostly to protocatechetic acid, and a few times also to *p*-oxybenzoic acid as sole carbon food.

After about a year, white, jelly-like spots were observed in a great number of the films floating in ERLENMEIJER-flasks of 200 cc. Seen under the microscope these spots proved to have produced but few spores, whereas the mycelium and hyphae were normally developed. The phenomenon became still more prominent if considerable quantities of other substances retarding the growth, such as salicylic and trichloracrylic acid were added to the *p*-oxybenzoic acid.

TABLE I.

50 cc. tapwater, 0,05 % NH_4Cl, 0,05 % KH_2PO_4, 0,02 % $MgSO_4$; $t = 20-21°$.

Nr	Carbon compound added.	13 days after inoculation
1	0,15 gr. p-oxybenzoic acid (0,3 %)	Aspect rather normal, only slightly mucous.
2	„ „ „ „	
3	0,15 gr. p-oxybenzoic acid + 3,5 mgr. salicylic acid.	Very mucous, most in 5, least in 3. In 4 and especially in 5 few spores.
4	„ „ „ „ + 7,3 mgr. salicylic acid.	
5	„ „ „ „ +12,2 mgr. salicylic acid.	
6	0,15 gr. p-oxybenzoic acid + 3,4 mgr. trichloracrylic acid.	6, 7, 8 successively like 3, 4 and 5, but the phenomenon less marked.[2])
7	„ „ „ „ + 7,1 mgr. trichloracrylic acid.	
8	„ „ „ „ +14 mgr. trichloracrylic acid.	

[1]) BOËSEKEN and WATERMAN. These Proceedings Vol. **14**, p. 604, 608, 928, 1112.

[2]) Salicylic acid retards the growth more than trichloracrylic acid.

It was supposed that the observed alteration in the mould film might be explained by mutation, which was proved true by the biological method. By isolation on malt gelatin two forms could be obtained from these cultures. One of these was very lightly coloured in consequence of the small number of spores. This form will be indicated as "the mutant". The other had preserved the dark green colour and had evidently remained identic with the original culture. The difference between the two forms was very marked.

So it cannot be doubted, that at prolonged cultivation in presence of *p*-oxybenzoic acid mutation does indeed take place. With protocatechetic acid as carbon food the same was observed. Furthermore, Table 1 shows that salicylic acid and trichloracrylic acid promote this process.

In the floating mould layer the extent of the mutant was greatest in those flasks where the said antiseptica were most concentrated.

At a continued cultivation on malt agar the thus obtained mutant, which in all the said cases seemed the same, remained constant.

If the mutant and the original form were again transferred to a *p*-oxybenzoic acid solution with the anorganic food named in the table, they also preserved their properties.

Under the microscope the mutant produced considerably fewer spores than the primitive form [1]) and its mycelium had a greater tenacity, which was repeatedly stated.

There was besides a peculiar difference in smell, as the original form gave out the well-known "mould odour", which the mutant did not.

The growth of the mutant on para-oxybenzoic acid was considerably slower than that of the primitive form.

In the laboratory a third form of *Penicillium glaucum* was present, distinguished from the original form of my experiments by darker green spores and which served for the subsequent experiments.

It was first cultivated during four days on *p*-oxybenzoic acid where the growth was very slow; it was then transferred to a new flask with the same medium, and now the growth was much accelerated, which proved that in these few days accommodation to the para-oxybenzoic acid had taken place. Furthermore it was observed that also here, after a prolonged cultivation on *p*-oxybenzoic acid mutation occurred. Substances such as tetrachlor-propionamid ($CHCl_2CCl_2C{=}O$ with NH_2) and pentachlor-propionamid ($CCl_3{-}CCl_2{-}C{=}O$ with NH_2),

[1]) Whether the difference in the number of spores was accompanied by a difference in intensity of colour is not settled as yet.

likewise compounds which retard the growth, again favoured the mutation, so that this process seems rather general.

The smaller number of spores and the less rapid growth evidently lead to explain the properties of this mutant by a loss of characteristics or gens. [1])

B. Aspergillus niger.

We started for this investigation from a pure culture of the laboratory collection, which was first cultivated some time on a 2 °/₀ succinic acid solution. In several inoculations in ERLENMEIJER-flasks with different culture media, a considerable alteration of this black mould occurred.

Using a 2 °/₀ solution of galactose it was observed that in this medium, beside the primitive form with black spores, a brown and a white one appeared, which three forms may be called I, II, and III.

On a 2°/₀ rhamnose solution of for the rest the same composition (tapwater, 0,05°/₀ NH_4Cl, 0,05°/₀ KH_2PO_4, 0,02°/₀ $MgSO_4$) the black and the brown forms (I) and (II) were distinctly present, the white form (III) wanting. A tube, to which beside the food consisting of 0.3 °/₀ *p*-oxybenzoic acid, 9 mgr. (per 50 c.c.) dichloracrylic acid $\begin{pmatrix} CH{=}CCl{-}C{=}O \\ Cl \qquad\quad \backslash OH \end{pmatrix}$ had been added, showed after about a month a quite brown mould layer. Later experiments proved that in nutrient solutions with 2 °/₀ glucose as source of carbon, under the influence of 1 °/₀ boric acid likewise mutation occurs.

The three forms from the galactose solution were isolated on malt agar; II and III distinctly gave fewer spores than I, and III fewer than II. They were transferred to media of tapwater-agar to which beside 0,05 °/₀ NH_4NO_3 and 0,05 °/₀ KH_2PO_4, 2 °/₀ galactose was added. On this plate the appearance of the mutants was different from that on the malt agar. From this galactose plate I, II, and III were again transferred to malt agar; the latter cultures were used for the examination of the plastic aequivalent of the carbon, whereunto we return below.

It was clear under the microscope that besides a smaller quantity of spores, there was also a decrease of colour intensity of these spores in II and III, which had become brown instead of black. The question whether III might also be obtained without any spores

[1]) Compare M. W. BEIJERINCK, Mutation bei Mikroben. Folia Microbiologica, 1912, p. 5.

at all must be answered negatively, as is shown by the subsequent experiments.

By starting every time from a single spore, cultures were obtained which remained identic to the material used for the sowing. If the mycelium, carefully separated from the spores was separately sown, no difference appeared between the product obtained from it and that from the spores.

Possibly form II is the same as the brown form obtained some months ago by FRL. SCHIEMANN [1]) under the action of kaliumbichromate.

In earlier experiments on the metabolism of *Aspergillus niger* irregularities had been found, which then could not be accounted for, but which can now be explained by the observed mutations. In the said experiments it was determined what percentage of the assimilated quantity of carbon was at a given moment bound in the body of the mould and what percentage was excreted as carbonic acid by respiration or otherwise. The first percentage may be called "*plastic aequivalent*" of the carbon, in accordance with the term used in researches on the luminous bacteria by Professor BEIJERINCK [2]) whereas the percentage of the carbon which at a given moment is respirated will be called "*respiration aequivalent*".

On a 0,3 % paraoxybenzoic acid solution (anorg. food : tapwater, 0,05 % NH_4Cl, 0,05 % KH_2PO_4, 0,02 % $MgSO_4$; t = 32—33°C.) was found after 45 days a plastic aequivalent of the carbon of **34** %. In other cultures likewise on para-oxybenzoic acid and obtained by inoculation with the said culture, whose plastic aequivalent was 34 %, this number amounted after 27—28 days respectively to **20** and **16** %.

As this lowering of the plastic aequivalent under the influence of the *p*-oxybenzoic acid might possibly be ascribed to the above mentioned mutation the question arose: Do forms I, II, and III quantitatively differ considerably in their metabolism?

The experiments resumed in Table II prove that this is really the case.

The differences are, as we see, enormous and they sufficiently explain the described irregularities.

By this method we are thus enabled to conclude to mutation even then when visible external differences between the cultures are wanting.

Likewise as for the mutation of *Penicillium glaucum* we see in

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[1]) Ber. d. Deutsch. Bot. Gesell. 1912 Heft 2, 28 März.

[2]) Aliment photogène et plastique. Archives Neérlandaises, T. 24, p. 1 1891.

TABLE II.

200 cc. ERLENMEIJER-flasks of Jenaglass with 50 cc. tapwater, in which 0.05 % NH_4Cl 0,05 % KH_2PO_4, 0,02 % $MgSO_4$ and 150 mgr. para-oxybenzoic acid, temperature about 32°—33°.

	Form I	Form II	Form III
Plastic aequivalent of the	29 %	18 %	15 %
carbon in two experiments	28 %	18 %	16 %

the here described mutation a loss of characteristics or gens, for beside the loss in colour intensity we stated a decrease in the number of spores.

On the other hand it was observed, that the new forms were distinguished from the primitive one by a much more vigorous combustion of the *p*-oxybenzoic acid to carbonic acid, their "respiration aequivalent" being found to amount from 71—72 % in I, to 82 % in II, and even to 85 % in III.

If, as in the case observed, all other carbon-containing secondary products are wanting, the sum of the two aequivalents is of course = 100.

The here introduced aequivalents only relate to the *element carbon*, whereas the hitherto used coefficients refer to the number of grams of dry substance, to the number of grams of assimilated carbon, or to the carbonic acid evolved during the life of the related organism [1]).

The here introduced aequivalents are to be preferred to the other terms referred to, because the chemical composition of the food, of the constituents of the organism, and of the carbonic acid are so widely divergent.

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1) See for instance: KUNSTMANN, Ueber das Verhältnis zwischen Pilzernte und verbrauchter Nahrung. Dissertation Leipzig, 1895. Also: NATHANSOHN, Stoffwechsel der Pflanzen, 1910.