PHYSIOLOGICAL STUDIES IN RELATION TO THE TAXONOMY OF MONASCUS SPP.

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A sporadic and destructive development of "mold" in the kilns of a maize starch factory, near Johannesburg, South Africa, in 1926, aroused sufficient interest to warrant an inquiry into the physiological characteristics of the fungus. The rich pink coloration of the starchy substratum and the microscopic appearance of the mycelium, led to its identification as a species of Monascus. Considerable assistance was given, in this connection, by Dr. Johanna Westerdijk and Miss Mes of the Bureau voor Schimmelkultuur, Baarn, Holland, to whom the writer is indebted not only for suggestions, but for cultures of related species. It was not then possible to come to any satisfactory conclusion about the specific name of the form, with the result that further work was carried out in the University of Wisconsin, as a subsidiary problem to a more detailed cytological study, the results of which will be published in a subsequent paper.

The genus was first described in 1884, by van Tieghem (20), from material on a boiled potato culture in France. At this time he determined two species: *M. ruber*, the type form, and *M. mucoroides*, with somewhat larger fruiting structures. Another species, erroneously regarded as *Physomyces heterosporus*, found by Harz (9) in glycerin solutions in a soap factory in Bavaria, was later correctly determined by Schröter. In 1895, Went described *Monascus purpureus*, which he obtained in Java, where, however, it was not originally grown. The Chinese cultivate the fungus on rice, with the result that the mycelium develops profusely until the rich crimson pigment has permeated the grains completely, when the whole mass is dried, cut into cakes, or ground to a greyish red powder. In this form it is exported throughout Eastern Asia, where it is known under the trade names Ang-qua, Ang-khak, Beni-koji and Aga-koji, and used extensively in the coloring of fish and other foods and in the preparation of spirits. This species
and the closely related *Monascus Barkeri* Dangeard are of additional commercial importance in the manufacture of Anchu and Samsu, alcoholic drinks. From Saito’s description it seems clear that Beni-koji is in reality composed of *Monascus purpureus* and a yeast, the former being capable of breaking down the rice starch into a simple sugar, while the yeast renders the further conversion into alcohol possible. The whole process, then, is effected by these two distinct organisms.

Went (22) first attempted an investigation of *Monascus purpureus*, both from the morphological and from the taxonomic point of view. He considered the fungus as belonging to the Hemiasci, in accordance with the views of van Tieghem and Brefeld, and his conclusions were supported in the main by Uyeda, working later with the same organism. It was Barker (1), however, a few years later, who secured material of the Samsu fungus from the Malay Peninsula; he made a careful cytological study of all stages in the life history, and established its identity as a true Ascomycete, although he regarded it as a very simple sexual type. Ikeno (11) and later Kuyper (13), working with the same species, strenuously opposed Barker’s views, the former continuing to regard the fungus as a Hemiascomycete, while the latter proposed to establish a new order, the Endascineae, for this genus, and to include it among the true Ascomycetes. Olive (16), supports Barker to some extent, but disagrees in regard to the perithecial initial; he feels convinced, however, that all the previous authors have been dealing with *Monascus purpureus* which seems justifiable, since all the material came from pigmented rice. Dangeard (6) regards the Samsu fungus as a distinct species without, however, giving any description of its individual peculiarities. Barker had found certain morphological and cultural differences between his form and *M. purpureus*, but he stresses the probability that Went was dealing with a fungus having a less specialized fruiting body. This explanation based on the wide divergency in interpretation of perithecial development by these two authors must be ignored in the light of Schikorra’s studies on *M. purpureus* Went, which will be considered below. In any case, the morphological characters, upon which species are largely based, would make *M. purpureus* and the Samsu fungus closely related species. A summary of the distinguishing features of *Monascus Barkeri*, will be given later.
In 1909 appeared the valuable cytological contribution to the life history of Monascus by Schikorra (19). Besides the investigation of *Monascus purpureus* Went, a comparison was made with another, “non-pigment”-producing, form from a fermentation institute (Gärtungsgewerbe) in Berlin. From his observations, and also from recent cultural studies, it seems probable that Schikorra’s *Monascus X* is a distinct species.

Reports of Monascus occurring in an unusual environment came from America in 1910, the first by Buchanan (4) from material causing mold in maize silage, the second from a bottle of pickles isolated by Lewis (15) and identified by him as *Monascus Barkeri* Dangeard. In the same year Piedallu (17) records a number of culture trials carried out in order to ascertain whether a form, which he collected from oil cans and skins from a tannery in France, was *Monascus purpureus*, as he had suggested in a former paper. After a comparison with Went’s species and with *Monascus Barkeri* Dangeard on various media, he concluded that his fungus differed from them chiefly in physiological characteristics, and referred it to a new species, *Monascus olei* Piedallu.

Since that time there have been no published reports of any further species, although in correspondence with Dr. Leva Walker of the University of Nebraska, it appears that the fungus, although somewhat sporadic, is fairly common on maize silage in the corn belt. Some herbarium material of moldy silage, several years old, kindly sent by her, has since been kept in cultivation in a flourishing condition.

It is evident that Monascus is a cosmopolitan genus, for its range has been extended not only to South Africa, as stated above, but an unpublished record by Charles McGee of two strains on maize silage was made in Australia in 1926. To him acknowledgments are due for material of these strains grown on silage agar. In any consideration of this extended range, it will be necessary to take into account the fact that various agronomic strains of maize have been exported from America to South Africa and Australia during the last two decades, and that contaminants must obviously have been carried along with the grain.
I. THE MAIZE STARCH MOLD

An inspection of the factory from which the moldy starch blocks came led at first to the impression that the dampness and the supply of carbohydrate and proteinaceous materials contained in wooden troughs were admirably suited to the growth of Monascus. In all sections of the building, however, where the germ is freed from the rest of the grain, and through the shaking and settling processes, the starch is in motion in SO₂ water (0.02 to 0.06% concentration). Only along the sides of the troughs, where splashing occurs, and on the soaking around the iron posts, were there evidences of pale pink masses of Fusarium sp. accompanied by a bacterial growth. In no case could Monascus be identified in these masses, in spite of repeated examination. As the starch settled and the gluten was diverted to another channel, the excess water was drained away and the mass was cut into blocks which were subjected to a blast of hot air at 96° C. in wooden-lined kilns. Any impurities (chiefly mineral) were then cut away with the outer crust, and the blocks were wrapped in heavy kraft paper. Up to this stage there was no opportunity for the development of mycelium, but here the blocks were placed in steam-heated kilns where the temperature rose slowly from 43° to 66° C., during a period of 14 days. The infected blocks were located in that part of the kiln which was farthest from the pipes, indicating some heat irregularity such that in regions where a temperature between 30° and 40° C. prevailed, an ideal environment was provided for the germination and development of the fungus.

In the kiln the remarkable development of the mycelium had prevented the normal “crystallizing” process; the starch dried out very slowly and became divided into prisms, so that the product was valueless, being loosely granular, and colored at intervals throughout by a delicate rose pink and spotted by innumerable perithecia. The individual starch grains were swollen and often burst, the ramifying hyphae very much contorted (fig. 1). The presence of a certain amount of glucose as a result of the fungal activity, along with the mechanical binding action of mycelium, held the block together.

Much of the waste starch lying in the region of the kilns, contained vigorous growths of Penicillium sp. and Aspergillus
sp., but no consideration was given to these, since better factory sanitation would prevent their serious development.

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After attempting to isolate the fungus directly from the block, it was found that far cleaner cultures were obtained if the moldy starch was washed overnight in running water. The individual perithecia could then be separated on a sterile slide in sterile distilled water, and broken inside the tube of agar before the plates were poured. The most successful nutrient medium was made by mixing 40 gms. of crushed maize seedlings with 600 cc. distilled water. This was used either as a nutrient solution, or combined with 12 gms. of agar as a solid substratum. Growth of the fungus is most rapid at 30°, 35°, and 40° C., although limited at the higher temperatures by the agar drying out very rapidly; cultures below 30° are slightly slower in development, and, below room temperature, considerably delayed. The lower temperature limit for growth was not determined. Conidia are produced after 16 hours and perithecial development is initiated within 48 hours. A deep crimson pigment appears within a week, increasing rapidly in older cultures. On darker media a purple tint appears in the red pigment, but rice cultures remain a vivid crimson. The pigment, however, fades fairly rapidly in the light, for freshly broken infected starch blocks, showing a rich pink interior, bleach out completely after some exposure. The mycelium is at first white, becoming gray in older parts in consequence of the prolific development of perithecia, which tend to appear in definite zones that are particularly marked on maltose agar.

In the nutrient solution growth is somewhat slower, but a spherical clump gradually appears in the liquid, which later turns crimson. Microscopic examination of this submerged mycelium reveals straight, vigorous, vacuolated hyphae, producing conidia rarely, and then singly at the tips of branches. This character is illustrated in figure 8; a portion of the mycelium having rich protoplasmic contents, numerous vacuoles, and almost no oil globules is shown in 8a; the formation of a single conidium in 8b; and a swelling, not completed as a conidium, but later extended to form two projections which will develop branches, in 8c. After some time, when the amount
of nutrient in the tube is reduced by half on account of evaporation, a portion of the mycelium develops on the surface, producing perithecia in that region, but not in the interior. Later work on the material of Monascus from Australia showed that similar vegetative growth occurs in 5% glycerin, until, when the liquid has evaporated considerably, perithecia are initiated all through the medium as well as on the surface. It seems evident, therefore, that there is a correlation between concentration of nutrient solution and perithecial formation, in accordance with the results obtained by Klebs (12) with oogonial development in a strain of Saprolegnia, and to some extent with those of Coons (5) on pycnidial development in Plenodomus fuscomaculans Sacc.

HUMIDITY

The somewhat rapid change in humidity in all these cultures under such warm conditions, as well as in the kilns, is suggested as a factor in the early formation of perithecia. On agar slants the growth is decidedly superficial at first, but later the mycelium develops toward the interior of the agar, this imbedded habit apparently being in response to the drying out under aerial conditions.

EFFECT OF PROTEIN PERCENTAGE

Several preliminary chemical analyses of moldy starch were made by Mr. J. A. McLachlan, the chemist associated with the factory, in order to ascertain whether the amount of protein present bore any relationship to fungal growth. The writer is gratefully indebted to him for these, as well as for the glucose determinations, for information concerning the technical aspects of starch production and for many helpful suggestions. His results showed that infected starch contained more than the minimum amount of protein, occasionally as much as 0.67%. Artificial cultures, in this connection showed the following:

(i) peptone agar—growth fairly good. Very slight pigmentation evidently associated with carbohydrate impurities in the commercial preparation.

(ii) pure gluten agar—growth poor, no pigmentation.

(iii) pure crystal starch agar—growth slight, clear rose-pink pigmentation.

(iv) coarse corn meal agar—growth good, red pigmentation.
No cultures having varying proportions of protein combined with carbohydrates were attempted, but cultural characters here and on maize seedling extract agar, as contrasted with pure starch agar, pointed to the beneficial effect of the presence of some protein. Further support of a proteinous medium playing some part was given in a second paper by Saito (18) on the enzymes of Monascus purpureus, where he showed that this fungus secretes a protein splitting enzyme, but no invertase.

TEMPERATURE AND OXYGEN SUPPLY

The fungus was shown to develop under a wide range of temperature conditions, and although the reading at the commencement of the last kiln process was 43° C., the following statements by McLachlan are of interest:

"The present design of the crystal kilns is such that uniform conditions of temperature and humidity are practically impossible, and this factor alone could account for the presence of mold in certain portions of a charge and not in others." The conidial and ascospore inoculum, evidently from infected grain, is present throughout, and resists the period of subjectio to hot air at 96° C. The fungus finds ideal conditions for germination in parts of the kiln where the temperature is slightly lower than 43° and an abundant supply of food material is provided.

No attempts were made to ascertain whether oxygen supply bore any relation to perithecial formation, as was suggested for Monascus purpureus by Went, although his experiments in that direction were not successful.

ACIDITY

With regard to the question as to whether the acidified water, which in the later processes reached a concentration of 0.1%, was efficacious as an inhibiting factor in the production of mycelium, several cultures were attempted. The fungus showed considerable tolerance to acidified liquid media and better growth on slants to which 1 to 5 drops of 1% SO₂ were added. Germination of ascospores took place in 0.025% SO₂ water, while conidia germinated in a 0.1% solution, but not in 0.15% SO₂ water.

Under factory conditions, then, control of the mold was ef-
ected most satisfactorily by increasing the acidity of the SO₂ water used in the various processes.

**SYSTEMATIC POSITION OF THE FUNGUS**

(a) **Description.** Mycelium from starch block contorted; in artificial culture, usually straight, hyphae 4μ, rarely 3 to 5μ in width, colorless, considerably branched in a monopodial or pseudo-dichotomous manner; conidia ovoid to pyriform varying considerably in size from 6 x 5μ to 16 x 14μ. (It may be noted that the length is given first in all cases.) Walls colorless or with a fair reddish light brown tinge; perithecia spherical 25 to 55μ in diameter, or subspherical 37 x 36μ to 50 x 42μ, walls colorless to light red brown; ascospores ellipsoidal 5 x 4μ to 6.5 x 4μ colorless, walls highly refractive. **Habit:** impure maize starch.

Growth on a variety of artificial media is easily obtained, but is luxuriant on potato-beef extract, dextrose agar, and corn-seedling extract agar (with 1 to 5 drops lactic acid added to each 5 cc. of medium). Pigmentation ranges from delicate pink in starch block, crimson on dextrose, dextrin and maltose agars, to deeper red, with slight purple tinge on dark colored media. Microscopic examination of milk culture shows presence of two distinct pigments, yellow and red, the latter predominating, but the yellow easily soluble in water. Range in temperature conditions considerable, optimum between 30 and 35°C, much delayed at 19°C, no growth beyond 45°C; in acid conditions, growth fair in 1% lactic acid, and SO₂ water up to 0.1% concentration.

(b) **Discussion.** According to the description, this species conforms most closely to *Monascus ruber* van Tieghem. There are, however, certain diverging characters: i.e., ascospores not more than 6.5 x 4μ, perithecia not strikingly red. A culture of *Monascus ruber* van Tieghem was obtained from the Bureau voor Schimmelkultuur, Baarn, Holland, labelled as such, but this fungus is almost identical with the starch mold, in particular, the ascospores are 5 to 6.5 x 4μ; the perithecia although usually red to red-brown, are often colorless; the range in size of conidia includes the measurements given by van Tieghem. If this specimen from Holland is exactly like the type form, then the size of the ascospores must have been exaggerated by
van Tieghem; on the other hand, it may be from a later collection, and identified as *Monascus ruber*. However, it seems evident that an authentic specimen of van Tieghem's type material does not exist. Although in culture the hyphae, conidia and even ascospores, to a slight extent, vary in size, the latter are approximately stable. Taking into consideration the deviation in size of ascospores and lack of any clearly defined red in the wall of the perithecium, in comparison with van Tieghem's fungus, there seems no justification for creating a new species for the starch mold which will be designated as *Monascus ruber* van Tieghem.

II. MAIZE SILAGE MOLDS

The two cultures of *Monascus*, mentioned earlier as having been sent from Australia, have shown consistent differences. The specimen from South Coast (sample E) forms a more vigorous mycelium which changes rapidly from a white cottony growth to dark grey, at 25°–35° C., in contrast to the Glen Innes material (sample F) where the growth is less conspicuous, paler, taking on a tinge of light brown as it ages. The morphological characters of both coincide with the starch mold, so that they are, therefore, specimens of *Monascus ruber* van Tieghem. The characteristic appearance of E is due to the excessive production of fruiting bodies whose walls range from colorless to light yellowish brown, but the interior is more often red, particularly in milk cultures. These perithecia vary considerably in size, spherical from 32 to 50μ in diameter or sub-spherical 32 x 30μ to 68 x 64μ; they are completely filled, in most cases, by ascospores whose colorless walls, by their highly refractive property, are largely responsible for the grey coloration. In culture and mycelial characters, F corresponds entirely with the specimen of *Monascus ruber* from Europe. Both fungi produce the vivid crimson pigment, without such definite additional yellow coloration appearing as in the starch mold. It may seem that pigmentation is stressed unduly, but these forms can readily be distinguished by this character. As an additional observation, the silage mold (sample G) from Nebraska always possesses a brown coloration, which gradually permeates the grey mycelium covering the strongly reddened nutritive medium, particularly in older cultures.
In consideration of the above statements, it is evident that the starch fungus and the silage molds, E, F, G, are separate strains of Monascus ruber van Tieghem, differing from one another biologically. The organism from silage, studied by Buchanan in 1910, is probably another strain of this somewhat variable species, for, although he suggests that it is Monascus purpureus Went, cultural characters of this latter fungus, combined with the nature of its conidia and ascospores, distinguish it from the silage molds. As in the case of the starch fungus, germination of conidia in strain E occurs in 6 hours. In figure 3 the germ tubes are shown issuing from a part in the spore wall, away from the region of attachment to the hyphal end. The cytoplasm is granular, rich in oil, and filled with small vacuoles. During the following 12 hours, septa are laid down in the elongating hypha, from which branches are beginning to develop, while conidia are formed in the older part (fig. 4). In cultures 48 hours old, particularly those grown at 35° C., conidia are present in abundance, often in long chains, which may be interrupted by short, unthickened portions of the hyphae, as in figure 5. When the fungus is cultivated on beef extract dextrose agar, a considerable development of chlamydo- spores results, although these may be found to occur upon other media to a slight extent. Portions of the hypha are separated by septa and, without any preliminary swelling, become thick- walled chlamydoospores. These may break away as do the conidia, but have not been seen germinating (fig. 6). Many of the perithecia in such cultures are small and contain only two asci (fig. 7).

DEGENERATION IN STOCK CULTURES

Particular interest has been centered in strain E of Monascus ruber, because its prolific perithecial development renders it admirably suitable for cytological study. Difficulty in arriving at the systematic position discussed above resulted in various attempts being made to secure Monascus cultures from Eastern Asia, but without success. It was necessary, then, to use transfers of M. Barkeri Dangeard, M. Purpureus Went and Schikorr’s form from the stock cultures kept at Baarn, Holland. All these, however, showed obvious signs of degeneracy, evidenced by complete lack of perithecia, irregular, desultory
conidial production and hyphae with disintegrating contents. This condition has been brought about by accumulation of toxic products in the cultures, resulting from a slight bacterial contamination occurring persistently in rice flasks. It is clear that the rice used was not sterilized, either by thorough autoclaving, nor by discontinuous sterilization for three successive days. This has caused considerable trouble in the culture work, and has necessitated the various known purifying methods being used.

In order to be quite sure of dealing with pure cultures, acid agars and solutions have been tried, since considerable acid toleration was shown by the starch mold, and as suggested by Brown (8) and also Hopkins (10). Monascus purpureus develops successfully on agar in which 1 or 2 drops of lactic acid have been added to each 5 cc. of the medium. When, however, 5 drops are added, growth practically ceases and the hyphae become bright yellow and filled with oil globules. There is no development in a 1% solution of lactic acid. This species forms conspicuous coils of hyphae on agars particularly those poor in food materials (fig. 2). These coils occur less frequently in silage molds. Monascus X Schikorra continues to develop a mycelium on ordinary media, but still without any fruiting bodies. It grows sparsely in 1% lactic acid, but well in 5% glycerin to which 1 drop of lactic acid has been added.

Monascus Barkeri Dangeard which in all solid cultures shows no signs of pigmentation, grows in 1% solution of lactic acid, also in acidified glycerin, where a delicate violet purple coloration has been observed. Only hyphae varying somewhat in width bearing infrequent single apical conidia are produced.

After the acid treatment, Monascus purpureus, when grown on potato dextrose agar, forms perithecia, so that it has been possible to compare this fungus both culturally and morphologically with the molds already discussed. Such satisfactory restorative vigor has not as yet been shown by either Monascus Barkeri or Monascus X Schikorra, so that it is impossible as yet to state with certainty whether this latter form can be raised to specific rank.

In order to determine the limit of acid toleration shown by strain E of Monascus ruber, a series of acidified distilled water (± pH 6) cultures of varying acidity were used. Conductivity water (pH 6) acted to some extent as a check and also served
to illustrate the minute quantity of food materials required by the fungus for the development of perithecia. Lactic acid solutions ranging from 0.05% increasing by intervals of 0.05% in the first case, and 0.5% in the remaining nine, were made up in sets—each set consisting of six tubes. The individual tubes contained 5 cc. of liquid. The cultures were placed in the incubator at 29°C.

The observations made during the succeeding period of four weeks are recorded in the following table.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Extent of mycelium</th>
<th>Pigmentation</th>
<th>Perithecia</th>
<th>Microscopic characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage: 0.05</td>
<td>$\frac{3}{4}$-$\frac{7}{8}$ in.</td>
<td>Slight pink coloration in gray mycelium, liquid uncolored.</td>
<td>++</td>
<td>Hyphae filled with oil; perithecia and conidia numerous, light colored.</td>
</tr>
<tr>
<td>0.1</td>
<td>$\frac{1}{4}$ in.</td>
<td>Rich pink coloration in gray mycelium mass, liquid uncolored.</td>
<td>++</td>
<td>Hyphae filled with oil; conidia and perithecia numerous, brown.</td>
</tr>
<tr>
<td>0.5</td>
<td>$\frac{7}{8}$ in.</td>
<td>Rich pink to red; liquid bright yellow</td>
<td>+</td>
<td>Hyphae filled with oil, walls slightly brown, conidia few, singly or in chains of 2. Perithecia rare.</td>
</tr>
<tr>
<td>1</td>
<td>$\frac{3}{4}$ in.</td>
<td>Rich pink to red; liquid bright yellow</td>
<td>+</td>
<td>Hyphae filled with oil, walls slightly brown, conidia few, singly or in chains of 2. Perithecia rare.</td>
</tr>
<tr>
<td>1.5</td>
<td>$\frac{1}{2}$ in.</td>
<td>Rich pink to red, liquid bright yellow</td>
<td>-</td>
<td>Hyphae similar, conidia few, no perithecia</td>
</tr>
<tr>
<td>2</td>
<td>$\frac{1}{2}$ in.</td>
<td>Rich pink to red, liquid bright yellow</td>
<td>-</td>
<td>At first hyphae richly vacuolate, with few oil globules. Conidia rare, single, distorted. Older cultures rich in oil, hyphae walls red brown, irregular contorted short branches. No perithecia.</td>
</tr>
<tr>
<td>2.5</td>
<td>$\frac{1}{2}$ x $\frac{3}{4}$ in.</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>''</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>''</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Slight, considerably delayed growth</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>None</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conductivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>water pH 6</td>
<td>$\frac{3}{4}$ in.</td>
<td>Grey coloration of the mycelium. No red visible, liquid uncolored</td>
<td>++</td>
<td>A small amount of mycelium with numerous conidia and perithecia.</td>
</tr>
</tbody>
</table>

Just as in the case of the starch mold, strain E shows a pronounced tolerance to acids. Transfers made from 3.5% lactic acid cultures on ordinary agars have provided the stock mate-
rial for microscopic work, since the fungus is now pure. Fresh mounts of this growth still continue to have minute bodies in the field within conidia and hyphae, exhibiting rapid Brownian movement. Staining with Sudan III shows them up as oil globules. These are evidently the bodies noted by Piedallu in his reference to minute bacilli in symbiosis with the fungus. Perithecia evidently develop in media varying considerably in amount of nutritive material. There is not only sufficient food supply in 0.1% lactic acid, but in conductivity water to produce fruiting bodies filled with normal ascospores. Although in acid solutions there is a limit to the concentration the fungus will tolerate, a vigorous growth is maintained in a considerable series of sugar solutions. For example, in maltose solutions, mycelium and fruiting bodies are found in concentrations ranging from 0.25 to 15%. In the higher dilutions there is no accompanying red coloration, but it appears and increases in intensity with the concentration of the medium; grey streaks all through the diffuse mycelial mass are the zones of perithecia. Extent of mycelial development increases with the concentration, but no cultures above 15% were tried in order to ascertain the extent to which this will occur. The optimum solution for perithecial development appears to be 2.5%.

A tendency towards degenerate types of growth in solutions of greater acidity is displayed by the mycelium, which is remarkably like that found in the stock cultures of *M. Purpureus* Went, *M. Barkeri* Dangeard and *Monascus X* Schikorr. The last three species are far less tolerant to acids than *Monascus ruber*, but in each case a growth occurs in media containing a certain concentration of a weak acid, but, when the quantity of dissociated H-ions is increased beyond the capacity of the individual fungus, a degenerate type of development results. This increase in H-ion concentration may be obtained by using a more concentrated acid solution, or may be derived, after a time, from the staling products of the fungus itself. The extremely slow growth of the bacterial contamination in some stock rice cultures points to the probability that the accumulation of excess acid, as a staling product from the mycelium, is an important factor in slowing up the further development of the fungus itself and also provides an environment too unfavorable for bacterial activity. It is well known that the production of staling substances is evidenced by a characteristic zona-
tion of the mycelium. This is strikingly shown in Monascus, where regular bands are sharply delimited by the presence of numerous perithecia. If Monascus spp. are kept in culture it is necessary to make transfers at reasonably frequent intervals, sometimes to a different substratum, but in the writer's experience material which can be kept in a dried form is far more satisfactory, an interesting case being that of Monascus purpureus which is handled commercially by the Chinese in the dried cake or powder form, where the ascospores remain viable for an indefinite period and are unaffected even when arsenic is added as a preservative.

CONCLUSION

Cultural studies of Monascus spp. have been of considerable value in the recognition of specific forms which are closely related. Of these, the silage and starch molds have shown some interesting physiological characters sufficiently distinct to warrant their being regarded as various strains of a single species, Monascus ruber van Tieghem, a description of which has already been given.

Monascus purpureus is characterized particularly by having ascospores which are usually spherical, being 5μ in diameter, or slightly ovoid, their size being 6 x 5μ. The youngest part of the mycelium is white, but it rapidly changes to a rich pink and later to a distinctly orange yellow, presumably as the environment becomes more acid, since this species is less tolerant to acids than Monascus ruber, and in an acid medium produces bright yellow hyphae. The pigment found in the substratum as the culture ages is deep crimson. Conidial production is infrequent.

Monascus Barkeri differs from M. purpureus in its prolific development of conidia, usually in chains, while the ascospores are ovoid, measuring 8 x 4μ. Although the present culture has shown no fruiting bodies, the cultural characters are distinct. A clear violet coloration appears in liquid media and the optimum temperature for growth is 25° C., (no development beyond 30° C.).

Monascus X is characterized by producing a vigorous pure white mycelium and, according to Schikorra, having larger ascospores than M. purpureus. No pigment is produced, ex-
cept in a few instances where a slight pink tinge may occasionally be discerned. Nothing further can be stated in regard to morphological characters since the culture is not fruiting, so that the question of whether this fungus is a distinct species must be deferred.

*Monascus olei* differs from the two previous species in physiological characters. These, alone, seem to provide insufficient grounds for the creation of a new species, but Piedallu appears to have made careful comparative studies before coming to any conclusion. His diagnosis must be accepted at this time, since it has not been possible to obtain cultures of the form.

*Monascus mucoroides* is also not available, but from van Tieghem's description, it is clearly a distinct species, characterized by the lack of pigmentation and the size of conidia, perithecia, and ascospores.

Lastly, it has not been possible to procure a specimen of *Monascus heterosporus* (Harz) Schröter, but in consideration of the pronounced variation in shape and size of conidia in *M. ruber*, there seems to be no justification for regarding the Harz form as a distinct species. This modification was suggested by Lafar in his description of this fungus, which is here definitely incorporated with *Monascus ruber* van Tieghem.

**Summary**

1. A strain of *Monascus ruber* van Tieghem causing moldy starch in South Africa, two strains from maize in Australia, and one from a similar environment in Nebraska, are reported.

2. The activities of the starch fungus cause, under certain conditions, the destruction of entire starch blocks. Luxuriant development occurs between 30 and 35° C., some growth at 40° C., but none at 45° C. A considerable growth occurs in 1% lactic acid and perithecial formation occurs in SO₂ water up to 0.1% concentration.

3. The temperature at which drying of the starch blocks is begun and the naturally rapid development of the fungus contribute to these sporadic and widespread occurrences, but the main factors are seen to be associated with the great tolerance of the fungus to acids and the vigorous growth in starch containing an excessive proportion of protein.
4. Of the silage molds strain E develops perithecia in greatest quantity; this fungus shows considerable acid tolerance, forming a mycelial growth in 3.5% lactic acid. Perithecia are produced on a variety of media, particularly those containing carbohydrates, but fruiting also occurs in 0.1% lactic acid and conductivity water.

5. The various species of Monascus degenerate after continued growth on artificial media, while dried material retains its vigor over a period of years.

6. Up to the present there appear to be five clearly defined species in this genus: *M. purpureus* Went, *M. Barkeri* Dangeard, *M. olei* Piedallu, *M. mucoroides*, and *M. ruber* van Tieghem.

7. *Monascus heterosporus* (Harz) Schröter) has been incorporated with *Monascus ruber*, which is a variable species, as shown by cultural studies, and includes a number of strains.

8. It will be necessary to obtain fruiting cultures of *Monascus X Schikorra* before it can be ascertained whether this is a distinct species.

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**BIBLIOGRAPHY**


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EXPLANATION OF PLATES

All drawings were made with the aid of a camera lucida, from fresh material.

PLATE 3

Fig. 1. Monascus ruber, as present in maize starch block, showing contorted mycelium, and broken perithecium, liberating ascospores. $\times 1280$ (approx.).

Fig. 2. Hyphal coil from culture of Monascus purpureus. $\times 736$.

Fig. 3. Germinating conidia, showing germ tubes, and flattened region of attachment to hypha. $\times 1175$.

Fig. 4. A culture 18 hours old, showing stages in development of branches at (a) and (b); the remainder of the original conidial wall is heavily outlined.

PLATE 4

Fig. 5. Portion of a mycelium in a culture 48 hours old, showing formation of conidia in chains of varying lengths.

Fig. 6. Chlamydospore production characteristically produced in the mycelium on beef extract-dextrose agar.

Fig. 7. A portion of the mycelium from the same agar culture, showing a small perithecium, with the trichogyne projecting downwards and the antheridium lying partly at the back, but extended into a branch bearing two conidia.

Fig. 8. A portion of the mycelium developed in dilute nutrient solution, showing at (a) rich vacuolate cytoplasm with no oil globules; at (b) a young conidium, and at (c) a swelling, which may have formed a conidium, but which has extended to form a branch, the apex of which is dividing pseudo-dichotomously.

(Figs. 3–8 inclusive are taken from strain E of Monascus ruber, and have a magnification of $\times 736$).