On *Avena sativa* 7, 1897 (King).

Puccinia Polygoni-amphibii Per.

Reported from Johnson and Putnam counties in 1893 on *Polygonium acre*, from Fulton and Wabash counties in 1893 on *Polygonium Muhlenbergii*, and from Tippecanoe in 1896 on *Polygonium erectum*.

On *Polygonium hydropiperiodes* 10, 1897 (King).

Puccinia podophylli Schw.

Reported from Johnson, Monroe, Brown, Owen, Vigo, Putnam, Montgomery, Wabash and Dearborn counties in 1893, and from Tippecanoe in 1896.

On *Podophyllum peltatum* 5, 1897 (King).

Uromyces caladii (Schw.) Farlow.

Reported from Vigo, Brown, Montgomery, Putnam, Monroe and Owen counties in 1893, and from Tippecanoe in 1896.

On *Arisæma triphyllum* 5, 1897 (King).

Uromyces trifolii (A. and S.) Wint.

Reported from Johnson, Montgomery, Putnam, Tippecanoe and Wabash counties in 1893.

On *Trifolium pratense* 10, 1897 (King).

The list of additional species to Tippecanoe county is small, only two new species having been found.

Æcidium Lycopi Gerard.

This species was found in swampy ground, and was quite abundant. The leaves and stems of the plant are covered with the *Æcidium* which eats holes in the leaves and destroys the host to some extent.

On *Lycopus sinuatis* 6, 1898 (Snyder).

Puccinia poarum Niels.

Found abundantly in lawns.

On *Poa pratensis* 5, 1897 (Snyder).

Aspergillus oryzae (Ahlburg) Cohn. By Katherine E. Golden.

A. oryzae is a mould which is of much practical interest by reason of its zymotic activity, since it secretes a diastatic ferment, and also for the claim which has been made that under certain conditions of growth, it is convertible into a yeast, and that, like most yeasts, it can give rise to alcoholic fermentation. It would constitute, in fact, if all claims made for it were true, a good working basis for an entire distillery. It has been used by the Japanese for centuries in one of their important fermentation industries, that of saké brewing, though like many other ferments used in early times, its true nature was not understood.

In the manufacture of saké, rice is steamed and then mixed with some rice which is covered with the mould, or else the rice is sown with the spores. The spores germinate in the moist and warm air, forming a much-branched mycelium which penetrates to all parts of the grains. This mycelium in developing secretes a diastatic ferment, which acts on the starch, first liquefying it, then changing the liquefied starch to sugar. The formation of spores is avoided by adding quantities of fresh grain from time to time, and mixing the fresh grain with that which has been inoculated. The addition of fresh grain is repeated several times, the mass thus formed of grains and mould being given the name "taka koji." The koji is mashed with about three times its volume of fresh steamed rice and four times its volume of water, and then allowed to stand at a temperature between 20° and 30°C. After some days the mash clears, from the saccharification of the starch, and a spontaneous fermentation sets in. This fermentation is due, however, to a different organism from A. oryzae. It is presumably on account of this fermentation that the mould has been erroneously called Japanese yeast. The fermentation goes on for two or three weeks, and at the end of that time the liquid is filtered. The resulting liquor is clear, pale yellow, and contains about thirteen per cent. of alcohol.

The mould has not been well known in this country until recently, though it has been known in Europe, and has received considerable attention from European botanists for about twenty years. In later years very enthusiastic claims have been made in regard to its physiological action, it being claimed that in the growth of the mould, "crystals" of diastase were formed on the filaments, that it was also so active and certain in its action as an alcoholic ferment, that in time it would entirely supersede yeast in the fermentation industries.

HISTORICAL.

The work of the first investigator, Ahlburg, in 1876, was the naming and description of the fungus. He called it Eurotium oryzae, because, as he said, the spores did not form chains, and the mycelium was not bent at angles. He described the sporangium as of a yellow color and possessing radiating spore tubes, and later on, in 1878, called attention to unimportant characteristics of the mycelium, thus indicating that he was uncertain in regard to the systematic importance of the various parts. He gave no illustrations, so that it was difficult to tell what he meant. In consequence of this, and that he named the fungus Eurotium, some of the later botanists interpreted the sporangia to be perithecia, and the radiating spore tubes asci.

Cohn, in 1883, in treating of the mould as an industrial factor in the manufacture of rice wine, speaks of it as A. oryzae, though he gives no morphological characteristics. Büsgen, in 1885, treats of the size and appearance of the mycelium, conidiophores, sterigmata, and conidia, though not very fully, as these were secondary considerations in his work. He also speaks of its resemblance to A. flavescens. Büsgen was the first to give a detailed description of the mould so that it was possible to compare it with other members of the genus. In 1893, Wehmer took up the work with the idea of making a detailed examination of the structure, and while he was thus engaged, Schröter's work in the same line appeared. Wehmer has a very careful, detailed description, and also some excellent drawings, and being a careful, conservative investigator, his work is particularly valuable.

Later workers are Takamine, Juhler, Jörgensen, Hansen, Klöcker, Schiönning, and Sorel. Takamine is a Japanese chemist who introduced the mould into this country for the purpose of its introduction into distilleries and breweries, his idea being to do away with the malting of the grain. This is to be effected by mixing the mould with the crushed grain in order to bring about the diastasic change in the starch by a less clumsy and more economical manner than the malting. He took out a patent in this country on the mould, his patent treating of its diastasic function and its transformation to a yeast. The method was introduced into a distillery and there Juhler obtained the mould, and took it to Denmark for study in Jörgensen's laboratory. Juhler claimed that the mould could be changed under certain conditions to a yeast, and Jörgensen indorsed him, and carried the assertion still farther by claiming that other moulds as well as A. oryzae possessed this property. Sorel got like results to those of Juhler and Jörgensen, but he makes a still further assertion in that he claims to reproduce the mould from the yeast when he sowed the yeast in a "not-quite-pure" condition upon the rice. The "not-quite-pure" condition undoubtedly accounts for his results.

Hansen took the opposite view in disclaiming alcoholic fermentation, and conversion of the mould to a yeast. Klöcker and Schionning, who worked in Hansen's laboratory, agree with Hansen's view of the matter, and this conclusion was arrived at after an extended investigation of the mould, this investigation including a repetition of Juhler, Jörgensen, and Sorel's experiments with original material furnished by Takamine, and also pure material. Wehmer in a second paper also agrees with the non-production of yeast and alcoholic fermentation, and states that there are two organisms that take part in the saké brewing, and that Takamine, Juhler, and Jörgensen did not discover the genetic relationship of the two. This, however, does not state the true condition of the case, for Jörgensen was aware of the two organisms present, and states that there is no genetic relation between the two.*

Other workers upon the mould are Atkinson of Tokio, who treated it from the industrial point of view, as did also Hoffman and Korschelt. Cohn, Büsgen, and Ikuta followed, their work being mainly along the same lines, though each one gave some additional information. The morphological and physiological characteristics were carefully worked out by Kellner and his assistants, Mori and Nagaoka.

There has been a large number of investigations made upon the life history of the fungus and yet there are some points left that are not clearly given, as the peculiarities of form due to varying conditions, and also the failure to take advantage from the industrial point of the power which the mould is said to possess of causing alcoholic fermentation. In an English medical journal the statement is made that the mould is capable of producing a strong and certain alcoholic fermentation, and is much more resistive to foreign organisms than is yeast; that for these reasons it would be much more effective and economical than yeast in the fermentation of bread.

MORPHOLOGICAL.

The material for the following experiments was some of the so-called "original" material, obtained from Takamine. This original material is a portion of koji, which was grown without any special precautions to keep it pure. Pure cultures were made from this material, and were also used.

*Jörgensen, A.; "Micro-organisms and Fermentation," p. 93, 1893.

A. oryzae is a mould of a yellowish green color when seen in the mature stage. The color varies with the age of the plant and also with the medium upon which the plant is grown. Favorable solid media are bran, rice, and wort gelatine. On rice and wort gelatine the young growths are of a light yellow green, the color being due to the numberless conidia formed. As the growth ages, the color changes to dark olive green. On plate cultures the mycelia are usually in colonies, due to the massing and germination of a number of conidia in one spot; as a result, the plate presents a very irregular growth appearance (photograph 1). On bran the color of the young growth is much darker than that of the same age on rice, and in old growths the color is brownish olive to dark brown. In very old growths not a trace of green appears.

The mycelium is a mass of fine, fleecy filaments, very much branched, and containing numerous septa. Wehmer states that the branching and septa were not easily seen, except with high magnification, but I had no difficulty in seeing both features with low powers, as photographs 2, 3 will show. These were taken from gelatine-plate cultures. The magnification is 75 diameters. In young growths the filaments are filled with a finely granular protoplasm, which becomes much vacuolated as growth proceeds (photographs 4, 5). The filaments vary much in diameter even in the same culture, the main filament being large, while the branches taper, sometimes these being extremely fine. In old cultures the filaments become very large, thick and rough-walled (photograph 6). They are always colorless.

The conidiophores can usually be distinguished from the mycelial hyphae as they gradually enlarge to the spherical end. The length varies to such an extent that any figures would not mean anything. The conidiophores are sometimes short branches at right angles to the filaments from which they arise sometimes so long that their connection is somewhat difficult to determine. Büsgen gives the length of the conidiophore as .5 mm., Schröter, 1 mm., while Wehmer merely states that they vary in length. They become much enlarged in old cultures, the walls become very much thickened and roughened (photographs 8, 9).

In young growths the sterigmata are short and regular, and vary from a few in number to sufficient to completely cover the spherical head; but in older growths, especially when submerged, they become septate, sometimes a sterigma developing into a conidiophore, which on its end again develops sterigmata. These peculiarities are found readily in moist chamber developments (photographs 10, 11).

The conidia are spherical and are formed by an abstriction from the ends of the sterigmata. They are colorless and smooth-walled when first formed and when grown submerged, but very soon develop a yellow color, which darkens to a green, and when old, olive and brown. As soon as the conidia developed in the drop in a moist chamber reach the air, the walls thicken irregularly and assume a fine warty appearance. Photograph 7 shows the submerged head, photograph 12, older conidia grown in the air. No pictures could be taken that give an adequate idea of the number of conidia formed in a chain, as in their growth they extend so far beyond the plane of the water drop that it was impossible to focus them. And again they are so lightly held together that any attempt to mount them under a cover-glass causes them to separate.

The formation of conidia is the only method of reproduction known; no perithecia have been observed, though they have been mentioned by the earlier investigators, but this has come about through the erroneous designation of the fungus as a Eurotium.

PHYSIOLOGY.

To determine if the mould were capable of causing alcoholic fermentation, the mould spores were shown in ten per cent. solutions of maltose. dextrose, lactose, and sucrose, also wort, all in fermentation tubes. No gas was generated nor was any alcohol formed. The mould, however, grew much better in dextrose and maltose than in lactose and sucrose. The lactose growth remained meager, but the sucrose was merely slower, finally reaching the same extent of growth as the dextrose and maltose.

To test the action in bread, cultures in wort were made of the mould and also of a yeast which gives a vigorous fermentation. After these had grown for five days, sponges were made in which the yeast and mould were used and equal quantities of the other ingredients. In one set the yeast was used alone, in another the mould alone, while in a third the yeast and mould were used together. The sponges were allowed to ferment, then kneaded into dough, and again fermented, at the end of which time they were baked. The yeast sponge fermented most vigorously, the yeast and mould much slower, while the mould sponge showed but very little change. The yeast and mould together took an hour longer than the yeast alone to reach the same degree of fermentation. The loaves from the yeast were of sweet taste and odor, and even-grained. Those from the mould were soggy and heavy, had a sweet odor, but left a sharp aftertaste. The loaves from the yeast and mould were very like those from the yeast, but also left the sharp aftertaste, though this was not unpleasant. Four persons having no knowledge of the constituents of the loaves, selected the ones made from yeast alone as being the best bread.

In testing the germinative power, cultures were made in wort, wort gelatine, Pasteur solution with the four sugars, lactose, dextrose, maltose, and sucrose from inoculating material that varied in age from very young through different periods to one year and eleven months, and which had been grown upon rice, bran, wort gelatine, wort, and Pasteur solution containing the different sugars. The results show that the germinative power lessened with age, but a more important factor than age was that of the original medium in which the culture had been made. Some of the growths from the wort gelatine plates had entirely lost their germinative power, while others were weakened. Wehmer states that the age of the inoculating material made no difference in germinative power, neither did the medium upon which it had been grown.

For ascospore formation young conidia were sown upon gypsum blocks in the usual way for obtaining yeast spores, and in about a month's time rounded masses of protoplasm, resembling yeast spores, were formed in some of the cells, though no cell-wall could be determined for these spore-like bodies. The same spore-like bodies were formed from the protoplasm in mycelial filaments undergoing the same treatment.

No experiments were made directly to determine the diastatic action, as work upon this has been done quite extensively by chemists.

In conclusion I would state that so far as any experiment would show, there was no indication that A. oryzæ has the power of causing alcoholic fermentation, nor of being transformed through any conditions whatever into a yeast. Neither can it be used effectively in bread-making.

LITERATURE.

Büsgen: "Ueber Aspergillus oryzae," Botan, Centralbl., No. 41, p. 62, 1885.

Jörgensen, A.: "Micro-Organisms and Fermentation," pp. 92-93, 1893; "Ueber den Ursprung der Alkoholhefen," Berichte des Gährungsph., Labor., 1895.

Klöcker, A., and Schiönning, H.: Experimentelle Untersuchungen über die vermeintliche Umbildung des Aspergillus oryzæ in einen Saccharomyceten," Centr. f. Bakt. u. Par., Bd. I, Nos. 22, 23, pp. 777-782, 1895; "Que savons-nous de l'origine des Saccharomyces?" Compte rendu du Laboratoire de Carlsberg, pp. 36-68, 1896.

Takamine, J.: "Diastatic Fungi and Their Utilization," Am. Jour. Phar., Vol. 70, No. 3, pp. 137-141, 1898.

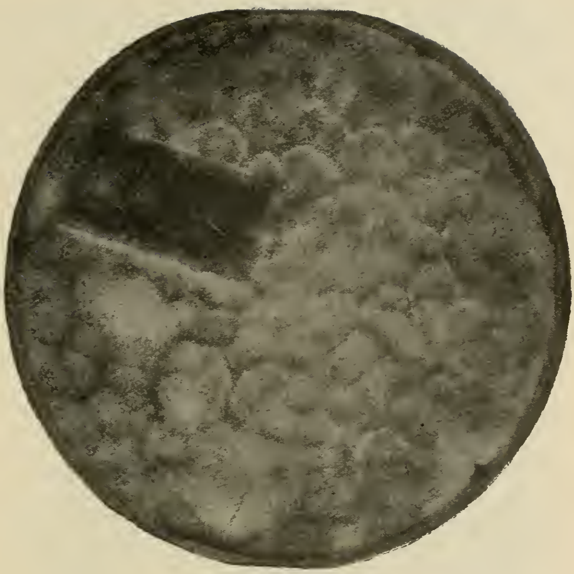
Wehmer, C.: "Aspergillus oryzae, der Pilz der japanischen Saké-Brauerei," Centr. f. Bakt. u. Par., Bd. I, Nos. 4, 5, pp. 150-160; No. 6, pp. 209-220, 1895. "Sakébrauerei und Pilzverzuckerung," Centr. f. Bakt. u. Par., Bd. I, Nos. 15, 16, pp. 565-581, 1895.

————: London Lancet, May 25, 1895.

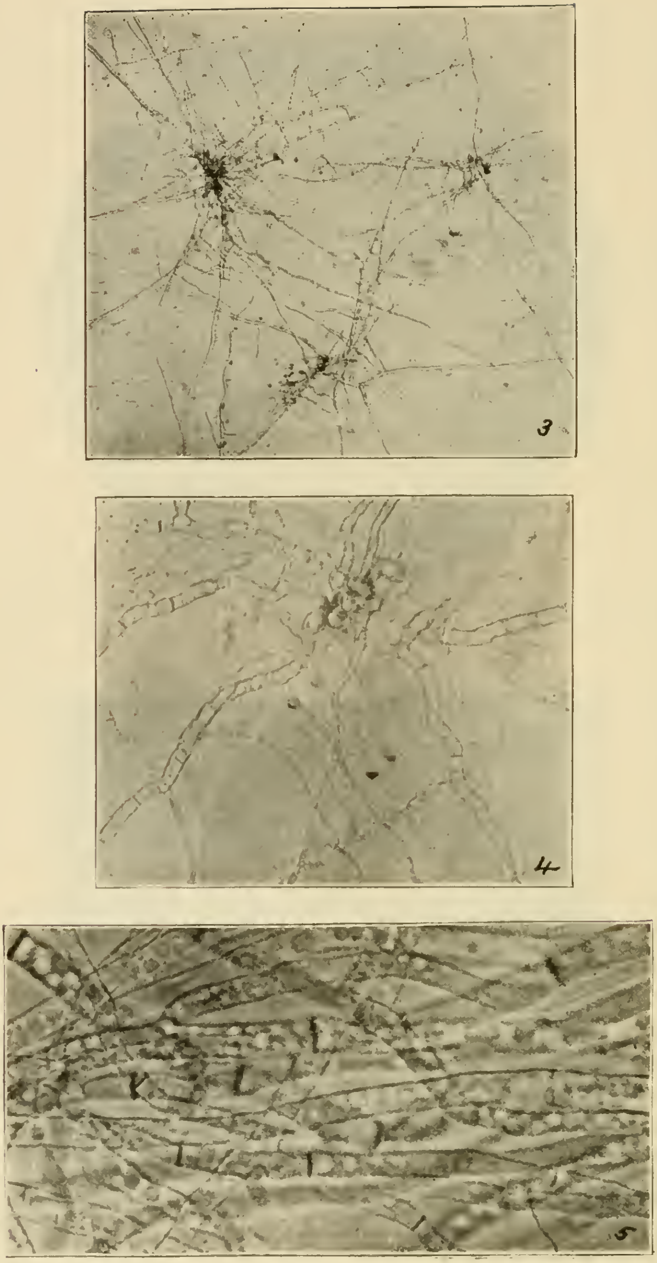
————: Boston Sunday Herald, September 15, 1895.

EXPLANATION OF PLATES.

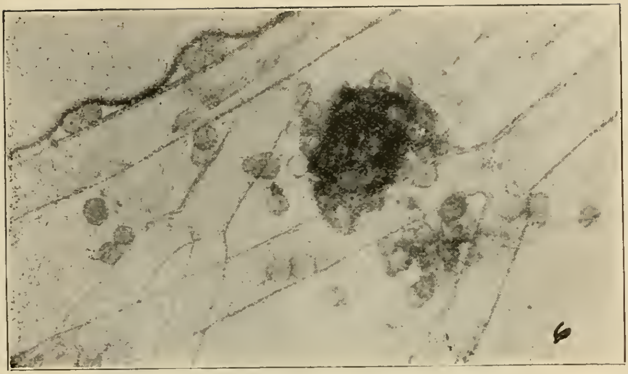
1. Wort gelatine plate culture, about a week old. ×⅔.
2. Germination of conidia in moist chamber. ×75.
3. Germination of conidia in moist chamber, in more advanced stage.
4. Same as 3, but higher magnification. ×495.
5. Filaments from wort gelatine plate culture. ×95.
6. Filaments from wort gelatine plate culture, ten months old. ×495.
7. Conidiophore grown in moist chamber, four days old. ×495.
8. Conidiophore from same source as 6. ×495.
9. Conidiophore from same source as 6. ×495.
10. Moist chamber growth, three weeks old. ×495.
11. Moist chamber growth, three weeks old, showing sterigma developing as conidiophore. ×495.
12. Spores from plate culture, three weeks old. ×495.



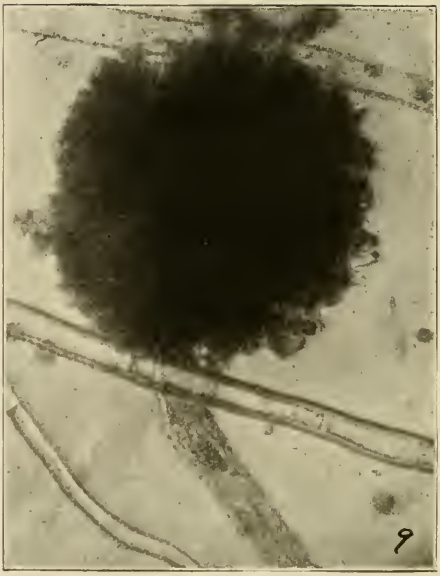
Golden on Aspergillus oryzæ.

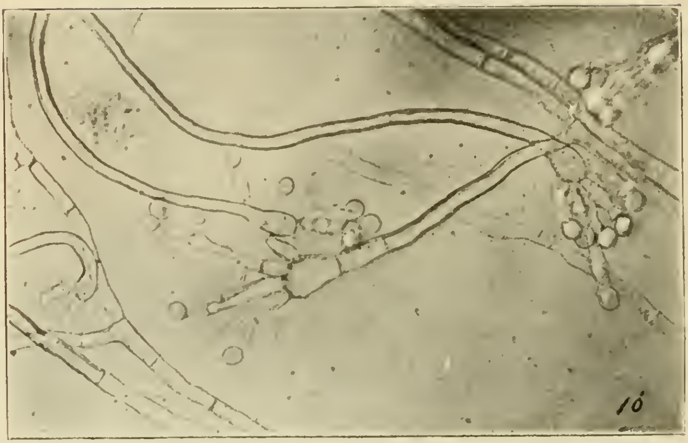


Golden on Aspergillus oryzæ.

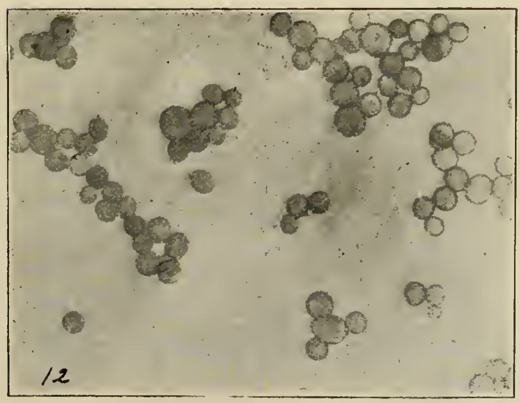


Golden on Aspergillus oryzæ.





Golden on Aspergillus oryzæ.



Golden on Aspergillus oryzæ.