

A Fragrant 'Mycoderma' Yeast, *Saccharomyces anomalus* (Hansen).

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With Plate XIII.
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AT the beginning of August 1899, during the course of a series of experiments on the fermentation of ginger, a greyish-white floury film was frequently found on the surface of saccharose-Mayer solutions¹ to which, after sterilization, pieces of commercial ginger had been added. Before the formation of this film active fermentation had been taking place in the liquid, and a slight growth of a species of *Aspergillus* and other organisms had appeared. Towards the end of active fermentation the film made its appearance and rapidly covered the surface of the liquid: about the same time a pleasant fruity odour was apparent, in some cases so strong that the whole laboratory was scented with it. The growth of the mould was stopped by the development of the film, because the latter prevented access of oxygen to the former. On examination of a portion of this film under the microscope, it was found to be made up of a number of small oval and round Yeast-like cells. The organism in its appearance

¹ Pure saccharose 15 grams. Mayer's Solution 10 c.c.

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and manner of growth seemed to be a form of so-called *Mycoderma*, with considerable resemblances in external characters to the ordinary *M. cerevisiae* and *M. vini*, but closer investigations showed that it is a very different organism.

Pure cultures were obtained by the following method:—a portion of the film was shaken up vigorously in a test-tube of sterile distilled water, and a drop of this liquid then poured into a test-tube of beer-wort gelatine¹. From this a fractional series of six plate-cultures of beer-wort gelatine was made and allowed to grow. Then a second series, infected from a colony growing on a plate of the first series, was made, to ensure the purity of the culture.

A stock of the organism was obtained by making a streak-culture on a tube of beer-wort gelatine from a colony developed on a plate of the second series. In order to prevent contamination of the stock, a new stock-tube of beer-wort gelatine was always infected whenever the original stock-tube was opened to obtain a supply of the organism, and the new stock used for the next supply. Occasionally a new series of plates was made, infected from the stock-tube in order to check the results, and to ensure that the organism conformed to type, and a new stock made from a single colony on one of the plates.

MORPHOLOGY.

In general culture on beer-wort, dextrose-Mayer solution, or other nutrient solutions², this Yeast forms, as a rule, a greyish-white film on the surface of the liquid. It has a very floury or powdery appearance, as if composed of innumerable greasy particles; and if the growth is very vigorous, the film becomes much wrinkled by mutual crowding of its units. Most of the cultures were made in Erlenmeyer flasks, and the Yeast had a curious habit of growing over the surface of the glass above the liquid up

¹ Unhopped Beer-wort 90 c.c. Gelatine 10 grams.

² See p. 230 for the constitution of these solutions.

towards the neck, like a greasy film on the moisture there found, the growth appearing somewhat mycelium-like in nature when undisturbed. A copious deposit of cells soon forms at the bottom of the flasks owing to gradual wetting and precipitation of the old cells: this deposit is white in colour at first, but becomes a dirty-brownish colour in old cultures. The liquid itself remains clear, when the film attains a good development.

CULTURE IN TUBES.

Streak-cultures were made on tubes of sloped beer-wort gelatine, and grew rapidly. A well-developed streak has in most cases a milky-white appearance. It is quite opaque, except that part of it which grows on the thin layer of gelatine at the upper part of the sloping surface, where the streak is semi-transparent and thin, and usually develops a mycelium-like fringe at its edge. The other portion of the streak is thick, moist, and fatty-looking, and its edges are more or less crenate and well-rounded. It does not grow down into the gelatine, but remains on the surface. In some cases when the gelatine dries owing to various causes, the streak has a different appearance altogether, being flat, glistening white in colour, eventually becoming dry and almost powdery, and marked with radial and concentric zones.

Beer-wort agar¹ has also been used, and the growth on this is even more rapid. Streaks similar to the fatty-looking streaks on beer-wort gelatine are produced.

PLATE-CULTURES.

On beer-wort gelatine. The forms of the colonies are by no means constant on different plates: on the same plate, however, as a rule they are fairly constant. This applies not only to plates of different series, but to plates of the same series, and will be explained below.

The colonies appear as small whitish dots. Those on the

¹ Agar 2 grams. Wort 90 c.c.

surface of the gelatine grow in the form of small domes, to about the size of a pin's head. Growth then takes place over the surface of the gelatine, and a thin flattened disc is produced. Sometimes it is circular in outline, glistening white in colour, and dry in appearance, marked by radial and concentric markings, and with regular edges (Plate XIII, Fig. 4). Sometimes it is like the preceding, except that it is semi-transparent, moister looking, more film-like, and with less regular edges. In other cases the colony is like an irregularly circular film, with crenate edges, semi-transparent, moist looking, with indications of radial and concentric zones (Fig. 3).

Colonies intermediate in character between the above forms have been observed. The form seems to be dependent on several different factors. The consistency of the gelatine has undoubtedly a great deal to do with the ultimate form. Plates were made with five per cent. gelatine instead of ten per cent., and the most usual form of colony produced in this case was the third described above. An abundance of moisture and higher temperatures (21–22° C.) tend towards the production of the same form. Colonies of the first form described have been observed to change into the other forms on increase of temperature.

On lactulose-Mayer gelatine the young stages of the colonies are similar to those on beer-wort gelatine. Instead, however, of the tiny dome-shaped colonies developing into flattened discs, as on beer-wort gelatine, they continue to grow in a dome-shaped manner, and in colour are glistening white. As they age, their surfaces are pitted with numerous tiny depressions, and at a later stage are fluted in a radial and concentric direction. They eventually become cone-shaped, or else develop a depression at the top of the dome. (See Figs. 1 and 7.)

In old colonies, as a rule, longitudinal splits are formed and they fall away into segments. Sometimes, however, they lose their white colour with age, becoming brown, and have a moist appearance, partially losing their shape. In a few

cases they changed to such a degree as to look like small drops of brown jelly. In other cases they changed into flat circular milky-white colonies, much resembling solid drops of stearin, and recalling the appearance of streak-cultures on beer-wort gelatine (see Fig. 8). All these later changes are due to slight liquefaction of the gelatine, accompanied by either a soaking in of the beer-wort, or some oxidation.

Plate-cultures made with dextrose-Mayer gelatine and saccharose-Mayer gelatine produce colonies of the same form as those in laevulose-Mayer gelatine.

Saccharose, *laevulose* and *dextrose* gelatine (relying on the traces of mineral substance in gelatine in the absence of other inorganic salts) were also used as media for plate-cultures. The colonies on each were similar in form. They grow as dots to the size of about a pin's head above the surface of the gelatine, but the growth is very slow, and then the formation of outgrowths like mycelia begins, and may continue until the central dot-like portion of the colony is surrounded by feathery radiations. See Fig. 6 showing colonies on saccharose gelatine.

General observations on colonies in plate-cultures.

The form of the colony seems to be dependent on the temperature, the amount of moisture, the crowding of the Yeast-cells and their vigour, the consistency of the gelatine, and the nature of the food-material. Old colonies have a tendency to produce mycelium-like outgrowths at their edges.

This matter would seem to be not without importance to the general question of the macroscopic appearance of colonies on plate-cultures. The typical form appears to be that of the raised, dry, chalky-white, brittle and even powdery wrinkled dome shown in the photograph (Figs. 1 and 7); but it is evident that any of the circumstances mentioned may so modify this that in extreme cases it would be difficult to recognize this organism from its plate-cultures.

Culture in hanging drops.

Cells grown under observation in hanging drops of beer-wort gelatine were ellipsoid or more or less ovoid in the adult stage. They were filled with clear, colourless protoplasm, and the extremely thin cell-walls were not distinctly marked off from the cell-contents. A single isolated cell may easily be overlooked on account of its transparency in the gelatine. At a temperature of 19–19.5°C. a single adult cell of the above form produced another similar adult cell in two hours by normal budding, the bud arising generally from the neighbourhood of the more pointed end. In the case figured (Fig. 9) the single cell had produced four similar cells in four hours, and in six hours seven cells. The daughter cells are at first round, but become usually more ovoid before producing another cell.

As a rule, after a colony of about eight cells was produced, the gelatine in the immediate neighbourhood was liquefied, with the result that the cells separated from one another, and the colony was thus broken up. Owing to the diminution of resistance as the gelatine softened, the buds separated from the mother cell much earlier than was the case when the gelatine remained unliquefied. These young separated buds bore a great resemblance to small *Torula*-like Yeasts. At a temperature of 15° C. the gelatine was not so rapidly liquefied, consequently larger colonies were found before separation, some containing a great number of cells. In hanging drops at this temperature about thirty-six hours old, the cells at the edges of the colonies have a tendency to grow out into a false mycelium, becoming longer and more rod-like in appearance. The colony then looks like a many-rayed star (Fig. 5), owing to the radiating series of branching cell-series to which I have throughout applied the term 'mycelium-like.'

In hanging drops a week old at 19° C. the cells have a sharply-marked cell-wall and are more or less vacuolated. Many of them contain bright refringent granules, and in some, spores were observed, as will be described subsequently.

FILMS.

*Primary films*¹ are formed by this Yeast on the surface of every culture-solution in which it is able to grow. They differ in character and in rate of growth according to the constitution of the culture-solution.

On beer-wort in most cases the film begins to form in twenty-four to forty-eight hours at 28° C. and twenty-four hours later appears as a thick greyish-white, dry and powdery, greasy looking, much wrinkled film. Fermentation then proceeds actively, and the film is to a great extent broken up by the bubbles of carbon dioxide evolved. When active fermentation ceases the film re-forms, but not so strongly as before, and after a time disappears and is replaced by the secondary film. In a few cases no continuous film was formed until fermentation ceased. At 18° C. the formation of the film took place more slowly.

On dextrose-Mayer solution a film is produced similar to that on beer-wort. It is however better-developed, more wrinkled and of a whiter colour. Formation begins in twenty-four to forty-eight hours at 28° C. Its fate is similar to that on beer-wort.

On saccharose-Mayer and laevulose-Mayer solutions the films are like those on dextrose-Mayer. On lactose-Mayer solution the first signs of film formation appear in about forty-eight hours after infection at 28° C. In its early stages it looks like a few patches of fatty matter lying on the surface of the liquid. These patches increase in size and eventually fuse together, forming a complete film over the surface in five days after infection at the above temperature. It is very thin and somewhat fatty-looking, and is also semi-transparent. It gradually breaks up and disappears.

On maltose-Mayer solution a film begins to develop in

¹ By primary film is meant the film which forms before fermentation, as distinct from the veils (secondary films), which Hansen found were developed by many Yeasts after the culture has stood for some time. (See 'Jørgensen, Mikroorganismen der Gährungsindustrie,' 4th ed., 1898, p. 173.)

about forty-eight hours after infection at 28° C. When fully developed it has a greyish-white colour, is very thin, and has a powdery appearance. It is not wrinkled, but resembles early stages in the development of the films on beer-wort and dextrose-Mayer.

On dextrin-Mayer solution after twenty-four hours at 25° C. the film begins to be visible. Twenty-four hours later it is thick, much wrinkled and of a grey colour, being identical in appearance with the films on beer-wort. It gradually breaks up and eventually disappears altogether.

On soluble starch in Mayer's solution a thin white powdery-looking film is formed in about seven to ten days at 28° C. The film is not usually well developed on the liquid, but spreads vigorously over the surface of the flask in a somewhat mycelium-like manner.

On examination under the microscope, the primary films are seen to be made up of Yeast-cells, actively budding, enclosing in the spaces between the cells numerous gas-bubbles. These bubbles adhere very tenaciously to the cells, and appear to be concerned in preventing the wetting and sinking of the film in water.

Secondary films are formed by this Yeast on those solutions in which it is capable of inducing alcoholic fermentation, viz. on beer-wort, dextrose, laevulose and saccharose-solutions. They begin to make their appearance some time after active fermentation has ceased, and after the primary film has disappeared. On cultures two months old the film can be observed as a thin fatty-looking layer over the surface of the liquid. It is semi-transparent and greyish in colour, and resembles somewhat in appearance the bloom seen on certain fruits, such as plums or grapes. The appearance of the film is the same on either beer-wort dextrose, laevulose or saccharose solutions. The films are formed both at room-temperatures and also at 25-30° C.

Under the microscope, numerous gas-bubbles are seen to be included among the Yeast-cells. Most of the cells are round or slightly oval with sharply-marked cell walls, one or

more large vacuoles, and bright refringent granules. Many, however, are elongated into pear-shaped cells, or rods: and small false mycelia, made up of these forms with a few round cells, are plentifully found. (See Fig. 10, *a. b. c.*, from secondary films on beer-wort, saccharose-Mayer, and laevalose-Mayer respectively).

FORMS OF CELLS.

The individual cells of this Yeast are of very different shapes and appearance, according to the conditions of growth, age of the cell, and other causes.

In young vigorous cultures the cells are almost entirely ellipsoidal or slightly egg-shaped, a few being round. They are filled with clear colourless protoplasm and are devoid of granules and vacuoles. The cell-wall is not sharply marked off from the protoplasm, and single cells on account of their homogeneity and transparency can easily be overlooked. This form is the actively growing form, and produces buds rapidly. Sometimes these buds are detached when still very small, this occurring principally in cultures in liquids; they are similar in appearance to the larger cells, but on account of their very small size look like small *Torula*-forms. The usual size of the cell at the time of budding is $5-7\ \mu$ in length, $4\ \mu$ in breadth. In old cultures this form of cell is rare.

When the cells just described have finished their active growth and division by budding, vacuoles begin to be formed in their protoplasm, and the cell-wall begins to be more sharply marked off from the cell-contents, the cell also becoming less transparent. Cells in this stage can be found in solutions that are actively fermenting. Later still, the cell-wall is sharply marked off from the rest of the cell, one or more bright refringent granules are formed, and the colour of the cell becomes somewhat brownish. Most of these cells are round, and in size vary from $3.5\ \mu-8.5\ \mu$. Some, however, have become elongated to a greater or less extent, and forms pear-shaped, rod-like filamentous, or thread-like are produced. The length of these sometimes is as much as $17\ \mu$, while in

breadth the pear-shaped forms are 8.5μ . Many of these elongated forms are found, placed end to end, forming a false mycelium. These cells with granules are to be found in old cultures, both in solutions and on solid media. They constitute almost the whole of the vegetation in these cases. Among them cells containing spores are usually to be found.

Illustrations of this type of vegetation are found in Fig. 10, *a. b. c.*, taken from secondary films formed on beer-wort, saccharose-Mayer, and laevulose-Mayer solutions respectively.

SPORES.

This Yeast is remarkable for the ease with which it can form spores and also for the shape of its spores. They are produced one to four in number in a single cell, the usual number being three or four. They are similar in form to those described by Hansen for *Saccharomyces anomalus*¹, being shaped like a half-sphere with a horizontally-projecting rim round the edge of the flat surface. From the resemblance to an ordinary 'Bowler' or 'Billy-cock' hat, they may be termed hat-shaped. In size they average 3.5μ – 4.5μ .

Cells containing spores are always to be found in old cultures, whether on solid media or in liquids. Spores which have escaped from their mother-cells are also abundantly found: those from the same mother-cell usually remain attached by their thickened rims to one another, and show the usual tetrad arrangement.

In order to obtain abundant supplies of spores the following method was adopted:—A few drops of a vigorous twenty-four hours' old culture in beer-wort at 28°C . were poured over sterilized pieces of porous biscuit-porcelain filter-plate. These were allowed to remain at 25°C . for forty-eight hours in a moist chamber, and on examination of the growth formed on the plate numerous spores were found. Abundant spore production takes place at any temperature between 18°C . and 28°C . Blocks of gypsum were tried instead of the porous filter-plate, but the latter gave the better results.

¹ Hansen (1).

The early stages of spore-formation have not been observed in detail, but there is no doubt that they are formed by repeated bipartition of the protoplasm in two successive planes, as in ordinary Yeasts.

GERMINATION.

For some time attempts to observe spores in process of germination were unsuccessful, apparently owing to my having used spores not yet fully ripened. Eventually success was attained by using spores developed on pieces of filter-plate, the growth on which had been allowed to thoroughly dry. Cells from the dried layer were well shaken-up in a tube of sterile beer-wort, in order to separate the individual cells completely, and then the tube was heated for five minutes at 55° C. By this procedure the vegetative cells were killed, and on making hanging drops of beer-wort gelatine the germination of the spores could be observed, without being obscured by the rapid growth of vegetative cells which would have taken place under ordinary circumstances.

Observations of the germination of several spores show that the process can be generally stated as follows: the spore begins to swell in about twenty-four hours after sowing at 18° C. The time that elapses before swelling begins is, however, very variable. During the swelling the spore becomes much more transparent than before. It swells until its diameter is about double its original size, viz. from 3.5 μ to 7 μ . A bud is then developed at some point on its surface, the position not being constant (Fig. 11). While this bud increases in size and attains the appearance of an active cell of this Yeast, one or more buds are developed at other points of the spore. When the buds attain the size of an ordinary cell, viz. about 5-7 μ , they in their turn produce buds, and thus a small colony is formed, the individual cells of which separate as the gelatine immediately surrounding them becomes liquefied.

The fate of the thickened rim of the spore has not been

clearly made out, but during the swelling of the spore it becomes much less noticeable. Whether it is a fold which stretches out as the wall extends, or a solid rim the substance of which swells and is used up, could not be determined. The buds are developed, not only on the rounded surface of the spore, but also on the flattened base, as seen in Fig. 11, so that the spore eventually loses to a great extent its hat-like shape.

It may be noticed as a point of interest that there is no preliminary mycelium nor any structure analogous to a pro-mycelium developed during germination, typical Yeast-cells being formed at the first budding.

PHYSIOLOGY.

Temperature limits. The optimum temperature of growth, as judged by the amount of development in streak-cultures on beer-wort agar-agar, is 28° C. Growth is possible at all temperatures between 15° C. and 32° C. At 10° C. it is very slow, while exposure for five minutes to a temperature of 55° C. kills all, or nearly all the vegetative cells. Exposure for the same period to a temperature of 50° C., however, leaves many cells still living.

Aerobism. Complete absence of free oxygen prevents growth, or at any rate prevents its initiation, but the presence of mere traces of that gas will allow growth to continue. Thus, if a flask of beer-wort infected with the Yeast be exhausted by the filter-pump for two hours, or even when the filter-pump is allowed to act continuously, the characteristic film is slowly formed on the surface of the liquid. If a series of plate-cultures be made, using beer-wort gelatine as the medium, and placed under the same conditions, colonies of the usual form are developed. This is probably owing to the impossibility of removing all the air by such means, because if the above experiments are performed in the presence of a solution of pyrogallic acid in caustic soda, in neither case is there a development of the Yeast.

Similar results are obtained if the inoculated flask is placed

in an atmosphere of hydrogen instead of *in vacuo*, the presence of pyrogallic acid and potash causing growth to cease, while in its absence growth takes place as usual.

These experiments seem to prove that the growth of the organism depends on the presence of free oxygen, since when the last traces of the free gas are absorbed by the alkaline solution of pyrogallic acid, growth is inhibited.

Other experiments bearing on the same point were made, using fermentation-tubes¹. These were filled with sterilized beer-wort and infected with the Yeast. It was found that when active fermentation was in progress, that gas frequently accumulated in the vertical closed tube. At first this was taken as indicating the anaerobic power of the Yeast, but this fact being at variance with the results of the experiments mentioned above, led to a repetition of the experiment. The same results were obtained when active fermentation set in, but it was noticed that the beer-wort in the vertical closed tube, instead of becoming cloudy, as one would expect if the Yeast were anaerobic and therefore were developing in that portion of the liquid, remained quite clear. The fermentation-tube was allowed to stand until active fermentation had ceased, and then it was noticed that the gas in the vertical closed tube was gradually absorbed again, until not more than one-twentieth part of the original volume remained. This was not absorbed, although the tube was allowed to stand for four weeks. The experiment was again repeated, and exactly the same results obtained. The explanation seems to be that at the beginning of active fermentation the evolution of carbon dioxide is so rapid that a portion of it strikes against the curved surface and rises by accident into the closed tube, and this is not absorbed until active fermentation has ceased, on account of the continuous evolution of the gas going on during that period. When this evolution stops, however, the liquid, if not fully saturated with the gas, gradually absorbs that portion contained in the tube.

Liquefaction of gelatine. The Yeast is capable of liquefying

¹ See Theobald Smith (23).

gelatine, but usually only to a slight extent. The amount of liquefaction that takes place varies considerably. During the autumn of 1899 apparently contradictory results were obtained from observations of the behaviour of plate-cultures of beer-wort gelatine and streak-cultures.

On some plates the gelatine was completely liquefied, while on other plates it remained quite solid: similarly also with streak-cultures. In some cases the gelatine in the neighbourhood of one or two colonies would be liquefied, while on the rest of the plate the gelatine remained solid. Thinking that these results might point to the presence of bacterial infection or of more than one variety of Yeast, the matter was tested by repeating the plate cultures, with the same results. Furthermore, hanging drop cultures from a single colony on a plate on which the gelatine had remained unliquefied were made, using beer-wort gelatine as the medium, the gelatine being in the proportion of ten per cent. The drops were kept under observation under the microscope, and the state of the gelatine in the neighbourhood of the Yeast-cells observed. It remained unliquefied until a colony of cells was produced, and then, in every case observed, it was eventually found to be slowly liquefied in the immediate neighbourhood of the cells, but the liquefaction did not in every case extend to the rest of the gelatine. Also the size of the colony, when the first appearances of liquefaction were observed, varied considerably. In drops four days old sometimes a colony was produced as large as a pin's head, quite dry and glistening white in appearance, and standing out above the surface of the gelatine. In such cases the gelatine was liquefied only in the immediate neighbourhood of the cells imbedded in the gelatine. In other cases the whole drop would be liquefied, and the Yeast would form a greyish-white film over the surface. These facts negative the idea of bacterial infection, since no Bacteria could be observed in the drops, and also the idea of a mixture of Yeasts of different properties, since cases in which drops were made from the same colony show the different results.

All the above experiments were carried on at the room-temperature, which varied from 15° C. to 21° C. on different days. It was noticed that the higher the temperature, the greater the tendency to complete liquefaction as a rule, although all colonies do not show the same energy. That the temperature itself is not solely responsible for the liquefaction is shown by the fact that when the temperature falls the gelatine does not solidify again, as would be the case if the higher temperature had caused it to melt. Hanging drops in a room, the temperature of which remains fairly constantly as low as 15° C., produced the glistening white dry colonies almost invariably, the gelatine being liquefied merely in the immediate neighbourhood of the cells imbedded in it: while hanging drop cultures in a room, the temperature of which was usually three or four degrees higher, showed much more variable results, complete liquefaction often taking place. Plate-cultures, generally speaking, show the same points. In warm weather they were often liquefied, and the usual form of colony was the film-like irregular form, while in colder weather liquefaction was comparatively rare, and the colonies were usually of the glistening white dry form.

The probable state of affairs seems then to be that the Yeast has a feebly developed power to liquefy gelatine, but that its power varies considerably according to the temperature, being much greater at about 20° C. than at 15° C.

These facts suggest that caution must be employed in deciding that a Yeast is capable of liquefying gelatine, since various circumstances—moisture, temperature, the state of the gelatine, crowding, and vigour of the cells, &c.—may continue to affect the question, just as in the case of the shapes, &c. of the colonies mentioned on p. 219, which in fact depend on the same properties.

FERMENTATION.

The capability of this yeast to induce alcoholic fermentation has been tested for the following carbohydrates:—xylose, mannite, gum acacia, dextrin, lactose, maltose, soluble

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starch, dextrose, saccharose, and laevulose. The solutions of each used, with the exception of soluble starch and laevulose, were 10 per cent. of the substance, made up with Yeast extract. Also with the exception of the first three substances mentioned, solutions of 15 per cent. of the carbohydrate made up with Mayer's solution were made, the constitution of each thus being:—

15	grams	carbohydrate.
1	gram	ammonium tartrate.
·5	„	potassium phosphate.
·25	„	magnesium sulphate.
·05	„	calcium phosphate.
100	c.c.	distilled water.

The action of the Yeast on beer-wort was also determined. The results showed that it can ferment saccharose (presumably after inverting it), dextrose, laevulose, and beer-wort, but cannot ferment xylose, dextrine, acacia gum, mannite, lactose, or soluble starch.

With reference to the fermentation of maltose, a few small gas-bubbles are found at the edges of the liquid, when film-formation has taken place. They are not numerous and may be due merely to air enclosed by the film during its formation. If they are due to fermentation, then the fermentation that takes place is very feeble.

A film is formed on each of the above solutions, but in the cases of xylose, gum acacia, and mannite, the growth observed may have been due to the presence of Yeast extract and not to the carbohydrate.

In all the other cases, however, growth was due to the carbohydrate, since cultivations on the substance dissolved in Mayer's solution produced films as well as those on the substance made up with Yeast extract.

Fermentation is of a vigorous character with each of the substances that the Yeast is able to ferment, that of beer-wort, however, not being as vigorous as the others. It started in from two to three days after infection at 28° C. and con-

tinued actively for several days. In the case of dextrose-Mayer solution, of which 250 c.c. were used in an Erlenmeyer flask of 500 c.c. capacity, the evolution of gas continued for two and a half weeks after fermentation had started, and saccharose and laevulose fermentations continued for about the same length of time. Beer-wort fermentations did not last quite so long, or in other words were completed more rapidly. At room-temperature, fermentation proceeded more slowly.

The characters of the vegetation on the different media used were similar, and the typical forms of cells found have been dealt with in the morphological portion of this paper.

PRODUCTS OF FERMENTATION.

Gases. The gas, collected in the closed vertical tube of the fermentation-tubes mentioned above when dealing with the aerobism of this Yeast, was analyzed. Nearly the whole of it was absorbed by caustic potash solution, thus showing that the bulk of it consisted of carbon dioxide. After the gas had been exposed to the action of potash for some minutes, pyrogallic acid was added, and a further portion was absorbed, this indicating the presence of oxygen. A small quantity of gas still remained unabsorbed and was presumably hydrogen or nitrogen. Stated roughly, the quantities of each present would be in every 100 parts 95 per cent. carbon dioxide, 1 per cent. oxygen, and 4 per cent. nitrogen, which makes it almost certain that the undissolved residue after absorption with potash is air which found its way in during the fermentation, probably from the air entangled among the cells of the primary films.

Ethyl alcohol. Fermented solutions of saccharose, dextrose, and laevulose were subjected to distillation and the iodoform test applied to the distillation. Ethyl alcohol was found to be present in each case. The amount present in the distillate, as estimated by an alcoholometer, was as much as 5 per cent. in some cases.

Higher alcohols are formed during the course of fermentation

in small quantities, among which amyl and butyl alcohols are predominant.

Organic acids. The fermented liquids show a well-defined acid reaction to litmus. Acetic, butyric, and succinic acids have been found.

Fruit ethers. One of the most conspicuous points in connexion with the fermentation brought about by this Yeast is the strong odour of fruit ethers that is developed after active fermentation has been in progress for some time. The exact odour varies, but that of ethyl acetate in some cases, and that of amyl acetate in other cases, are predominant. The odour in beer-wort fermentations is not as strong nor as well defined as in the cases of dextrose, saccharose, and laevulose.

HISTORY AND BIBLIOGRAPHY.

In 1891 Hansen (1) published a paper, *Sur la germination des spores chez les Saccharomycètes*, in which he gave a short description of a new species, belonging to that genus, which he named *S. anomalus*. The organism was characterized by an extremely quick film-formation on beer-wort, the film appearing at the beginning of fermentation, by the production of a strong odour of 'fruit-ethers' during the course of fermentation, and by the resemblance of the organism, when viewed under the microscope, to certain *Torula*-forms. During fermentation a strong odour of 'fruit-ethers' is produced. It was capable of forming spores under similar conditions to those required by other species of *Saccharomyces*, and they were also to be found plentifully in old cultures.

They differed from the spores of all other species of *Saccharomyces* in their shape: they were hemispherical, with one surface flattened, and they had a projecting rim round the edge of the flattened surface. Hansen called them 'hat-shaped,' and pointed out that they were identical in form with those of *Endomyces decipiens* (18). On germination they gave rise to no pro-mycelium, but, instead, produced buds similar to those developed by ordinary vegetative cells. The paper concludes with a short reference to the possibility of

genetic relationship between this new species and *Endomyces decipiens*.

In a paper published in 1892 Lindner (2) mentions a Yeast with hat-shaped spores. Jörgensen (3) also, in his book *Die Mikroorganismen der Gärungsindustrie*, gives a description of *Saccharomyces anomalus*, and mentions that other observers had found Yeasts with hat-shaped spores, which, apparently, were very closely related to the form described by Hansen, if not actually identical with it.

Ludwig (4) also describes the species. Fischer and Brebeck (5) have investigated an organism, which they have named *Endoblastoderma pulverulentum*, and which they state to be identical with the form *Mycoderma cerevisiae* (var. *pulverulenta*), Beyerinck. It was obtained from the lager-bier of a Rotterdam brewery. They distinguished it from the other species of their genus *Endoblastoderma* by its hat-shaped spores and by the pleasant fruit-like odour produced during fermentation. On beer-wort at 27°C., a white floury film was formed during the second day after infection. On beer a yellowish-white, thick wrinkled film appeared in the course of the first week.

Microscopically examined in young cultures, the cells were mostly round or egg-shaped, and strongly refractive. In older cultures the cells were of very various sizes, and occasionally false mycelia were found. The cells of the films were able to resist a temperature of 80–85°C. when exposed to it for ten minutes. Exposure, however, to a temperature of 60°C. for half an hour sufficed to kill all the cells. The colonies produced on plate-cultures reached a size of 5 m.m. in diameter and lay above the surface of the gelatine. In form they were flattened domes, circular in outline, white, dry, and pitted. They liquefied the gelatine substratum towards the end of the second week. Streak cultures produced complete liquefaction in seven weeks. Before liquefaction the streak-cultures showed a thick white layer, whose upper surface appeared as if it had been strewn with flour. The spores were hat-shaped and were formed usually in threes. The organism fermented beer-wort, dextrose, lae-

vulose, maltose, and saccharose; the fermentations, except in the case of laevulose, were very vigorous. Their genus *Endoblastoderma* was based on the property of endogenous cell-formation by its members, and they claimed that this manner of formation had been observed by them in the case of the organism just described.

Briefly stated, the method of endogenous cell-formation was as follows:—in a young cell a refractive particle made its appearance, and increasing in size made its way bodily through the cell-wall: it then developed in exactly the same manner as a bud produced in the ordinary manner by constriction from the mother-cell. The authors pointed out the great similarity of their organism to Hansen's *Saccharomyces anomalus*, and laid stress on the fact that the exact relationship could only be determined by testing the power of the latter to form cells endogenously in the manner just described. Klöcker (6) undertook this work, and failed to discover in *S. anomalus* any trace of endogenous cell-formation apart from the ordinary spore-formation. He noticed, however, the appearances on which Fischer and Brebeck based their view that endogenous cell-formation took place, and found that they were due to ordinary budding, the buds in some cells being produced in a direction more or less vertical to the cover-slip. On growing, these buds shifted their position in such a way that they appeared to move from the inside of the mother-cell to its exterior. Similar results were also obtained by him, when a variety of *Mycoderma* was investigated. There appears thus to be no ground for Fischer and Brebeck's construction of the new genus, *Endoblastoderma*. Klöcker stated in his paper that there could be no doubt that *S. anomalus* and *E. pulverulentum* are identical, from the description given of the latter.

Nielson (7) has investigated the effects of temperature on spore-production by *S. anomalus*. At temperatures above 33° C. no spores were formed. At 30° C. they made their first appearance in seventeen to nineteen hours; at 28° C. in seventeen and a half to nineteen hours; and at 25° C. in

eighteen to twenty hours. At $7\frac{1}{2}$ –6° C. thirteen to fourteen days were required before signs of spore-formation appeared, and at $2\frac{1}{2}$ –3° C. no spores were produced.

In Hansen's work (8) on the duration of life of various Yeasts under different conditions, it is stated that *S. anomalus* was able to live for more than eighty days, when spread in thin layers on a platinum wire needle. Most Yeasts died in five to twenty days.

Wehmer (9), in his studies on the capability of various Fungi to liquefy gelatine, found that *S. anomalus* did not liquefy 10 per cent. beer-wort gelatine. At the same time he draws attention to the fact that the form described by Fischer and Brebeck (5) under the name *Endoblastoderma pulverulentum* peptonized the gelatine.

Von Schukow (10) found that *S. anomalus* produced very little fermentation in unhopped beer-wort at 20–22° Réaumur; the fermentation was only about a quarter as vigorous as with many beer and wild Yeasts. He suggested that this was due to the fact that only the dextrose contained in the wort was capable of being fermented by the species.

S. anomalus has been met with in saké fermentations. Its presence has been noted by Klöcker and Schöning (11) and by Shieweck (12). The latter observer thinks that in conjunction with other Yeasts it plays an important part in the fermentation. This is very probable, since a strong odour of pine-apples is developed during the course of fermentation in the saké. This odour may be due to the formation of ethyl butyrate, and it has been shown above that the variety which I have investigated produces butyric acid. Yabé (13) has found a *Mycoderma* yeast growing on saké rice. Two other papers on *S. anomalus*, one by Steuber (14) and the other by Kujawski (15), have been recently published, but I have not had the opportunity of seeing them.

From a comparison of the various statements quoted above, of the different observers who have investigated the characters of *S. anomalus*—and we may accept Fischer and Brebeck's *Endoblastoderma pulverulentum* as a form of this species—it

appears that the distinguishing features of the species are the *Mycoderma*-like habit and the hat-shaped spores. Apart from these there seems to be considerable variability in the characters which have been noted. For instance, Fischer and Brebeck found that their organism ferments beer-wort, dextrose, saccharose, and maltose actively, while the fermentation of laevulose is less vigorous. Von Schukow found that his organism was able to produce only a comparatively slight fermentation in beer-wort, and accounts for it by supposing that only the dextrose is fermented. Certainly if the Yeast had been able to ferment maltose, a much more vigorous fermentation of beer-wort would have been expected.

The variety that I have examined ferments dextrose, laevulose, and saccharose actively, beer-wort less actively, and maltose in a very slight degree, if at all.

Different results, also, were obtained in connexion with the question of the liquefaction of gelatine. Fischer and Brebeck's organism liquefied gelatine completely; Wehmer's produced no liquefaction; while that described by me showed the variable behaviour that has been fully dealt with above.

Fischer and Brebeck found that exposure to a temperature of 80° C. for ten minutes did not suffice to kill the cells of their Yeast. Exposure to a temperature of 55° C. for five minutes was sufficient to kill the vegetative cells of my Yeast.

With regard to the number of spores formed by a single cell, Fischer and Brebeck found that three was the usual number. In the cases examined by me, although while the spores still remained within the mother-cell wall the number appeared to be three, yet probably four were present in most cases, the fourth being obscured by the positions of the other three: for when the spores had escaped from the mother-cell they were found chiefly in fours, grouped in tetrad arrangement, the fourth spore only coming into view as the group revolved in the liquid in which they were mounted.

The lack of detailed descriptions of the Fungi with hat-shaped spores¹ that have been found at various times renders

¹ *Ascoidea rubescens* (Bref.) also has similar spores. See Brefeld (18).

a complete comparison impossible, and conclusions as to the identity or relationship of the Yeasts can only be surmised, though they are extremely probable. It does seem probable, however, that there are differences between some of the forms, at least as great as there are between the different varieties included in the species, *S. cerevisiae* or in *S. Pastorianus*. It is interesting to note the wide distribution of these forms, some having been found in Japan, others in various parts of Europe, while quite possibly the form described in this paper is a West Indian form, the ginger on which it was found having come from Jamaica. Their wide distribution alone would lead one to expect considerable variety in their characters. The most satisfactory way of grouping them seems to be the inclusion of each form in the species *S. anomalus*; this species to have as its distinguishing characters the *Mycoderma*-like habit and the hat-shaped spores; and the subdivision of this species into varieties according to the behaviour of the Yeast in such points as the power of fermenting the various carbohydrates, the usual number of spores produced, the nature and rate of production of primary films, the occurrence and appearance of secondary films, the power of liquefaction of gelatine, the production of fruit-ethers¹, &c. The species would then have the same significance and would show a similar amount of diversity among its members, and yet be just as well defined as the better known species of *Saccharomyces*, such as *S. cerevisiae* and *S. Pastorianus*.

ON THE QUESTION OF RELATIONSHIP BETWEEN
S. ANOMALUS AND *ENDOMYCES DECIPIENS*.

In the paper by Hansen (1) quoted above it will be noticed that he draws attention to the possibility of genetic connexion between *S. anomalus* and *Endomyces decipiens*, on account of the similarity in shape of the spores of the two species, bearing in mind at the same time the position held by the latter as one of the simplest types of the group *Exoasci* and

¹ Beyerinck (21) describes other species of Yeast which produce fragrant fruit-ethers, *S. fragrans* and *S. acetasthylicus*, apparently quite distinct from *S. anomalus*.

the generally accepted classification of *Saccharomyces* with the Ascomycetes. In connexion with this question it is of interest to note the conclusion arrived at by Hansen (16) on the possible genetic relationship between *S. Ludwigii*, which in many respects shows a close connexion with *S. anomalus*, and another species of *Endomyces*, viz. *E. Magnusii*. These forms were discovered by Ludwig (17) in the 'Schleimfluss' of an oak, and this observer speaks with great reservation on the important question of their relationship. Brefeld (18) further investigated the form *Endomyces Magnusii*, and Hansen (19) has published a full account of *S. Ludwigii*. Both these observers agree in the conclusion that there is no clear evidence of undoubted genetic relationship between the two forms. I may also point out that recent attempts to connect the genus *Saccharomyces* with mycelial Fungi have broken down under the experimental criticism of Klöcker and Schöning (22).

Returning to the consideration of the other species, Brefeld (18) has given a description of *E. decipiens* in his account of the Exoasci, and also numerous figures of the species. The form is found as a parasite on the lamellae of *Agaricus melleus*, and consists of a branched mycelium, composed of elongated cells placed end on end. On the older parts of the mycelium asci are borne. They arise as short side-branches, which swell up and produce in their interior four hat-shaped spores. These spores are capable of germination immediately after ripening. In nutrient solutions they swell up, lose their original form, and from one or more points short tubes are developed which quickly grow to a branched mycelium. After two or three days the mycelium begins to break up into oidia. In the course of culture mycelia are produced, which are nothing more than long chains of oidia. Later, also, chlamydospores are produced singly on short side branches of a mycelium. They consist of a single large cell, with a yellow and thickened cell-wall, and contents rich in fat. Brefeld points out that they are morphologically equivalent to the oidia. On culture-solutions a thick white

film is formed, which originally consists entirely of oidia, but later chlamydospores appear and give to the film a yellowish colour.

On purely morphological grounds it will be noticed that there are certain resemblances between the two forms, but with reference to the mycelial formations, it would appear that here there is a distinct difference. Fig. 12, however, taken from the edge of a colony of *S. anomalus*, shows that there is a great tendency in this organism to produce a mycelium on a solid and dry medium. The asci in the two cases present considerable differences however. In *S. anomalus*, as far as we know, an ordinary vegetative cell becomes developed into an ascus under suitable conditions, while in *E. decipiens* the ascus is developed from a side-branch of the mycelium, i.e. in a somewhat definite position. This distinction, however, must perhaps not be pressed too much until definite knowledge is obtainable as to the power of every vegetative cell of the Yeast to form ascospores, and it is conceivable that it points merely to a slightly more specialized condition in the case of *E. decipiens*. The ascospores of both forms resemble one another completely in shape. Each ascus in *E. decipiens* typically produced four spores, while four is the most usual number in the form of *S. anomalus* which I have described. The germination of these spores, however, differs in a marked degree owing to the formation of a pro-mycelium by *E. decipiens*. The question may be raised whether this difference is as important as it appears at first sight. Hansen (1) has shown that the spores of *S. Ludwigii*, a species which is nearly related to *S. anomalus*, produce a sort of pro-mycelium on germination, while Reess (20) pointed out that the spores of *Taphrina Pruni* (Tul.) germinate in the same way as ordinary *Saccharomyces* spores, i.e. by budding. Brefeld (18) has shown the same for *Taphrina rhizophora* (Johans). The genus *Taphrina* is usually regarded as related to *Endomyces*.

From these examples, then, it does not follow that the production and non-production of a pro-mycelium are facts

which preclude the possibility of close relationship between two forms.

It might be argued that the formation of oidia by *E. decipiens* has a parallel in the case of *S. anomalus*, in the production of the rod-like cells, mentioned above as occurring in old cultures, and some of the forms of oidia figured in Brefeld's work are not dissimilar in appearance from the rod-like cells of *S. anomalus*, the 'mycelioid' forms of which, moreover, might be consistently regarded as series of oidia, if it were not for the fact that they are developed by budding. Nevertheless, the morphological equivalent for *S. anomalus* of the chlamydospores of *E. decipiens* has not yet been definitely determined, and even if it seems probable that the brownish-coloured large rounded cells, with well-defined cell-wall and fat globules, of the latter are practically chlamydospores, we can scarcely go so far as to argue their morphological identity. However, Hansen (8) has mentioned the power of resistance by the cells of *S. anomalus* to unfavourable surroundings, and it has been seen that in old cultures, for instance, the cells acquire the characters just described. In the case of the Yeast, any cell seems to be able to acquire the characters of a chlamydospore in a certain sense, but in *E. decipiens* these spores are usually developed in more or less definite positions, that is to say at the ends of side branches. Attention, however, must be drawn to the fact that Brefeld figures (Plate I, Fig. 27, 2, in his work quoted above) a chain of oidia, apparently not developed in any special position, which were acquiring the characters of chlamydospores. Hence the ordinary oidial cell of *E. decipiens* can be looked upon morphologically as a chlamydospore, thus corresponding in this capacity to the ordinary vegetative cells of the Yeast.

To carry the resemblance between the two species still further, it might be insisted that both form white films on the surfaces of nutrient solutions, and the older films of both show the presence of chlamydospores or cells that are equivalent to chlamydospores.

The property of inducing fermentation possessed by *S. anomalus* is absent in the case of *E. decipiens*. Perhaps this difference in behaviour need not be insisted upon as indicating a wide gap in the relations of these forms, for the behaviour of the various species of *Saccharomyces* with regard to different sugars, and even, as we have seen, the behaviour of different varieties of *S. anomalus* also, is so variable that it cannot be held to affect relationships higher than varieties. Still we find such films in many Fungi, and so cannot push the argument.

From this comparison of the two species, while there is no ground for supposing that *S. anomalus* is in any way a form of *E. decipiens* that has taken on a more or less permanent Yeast-like habit, yet there are indications of a relationship between the two species, and possibly a case could be made out for the view that the *Saccharomycetes* had their origin from the Exoasci, with such forms as *S. anomalus* and *E. decipiens* as connecting links, *Ascoidea rubescens* also, with considerably more divergent characters, being taken into the purview. As a matter of fact, however, no proof of the direct connexion between this or any true Yeast and a typically mycelial fungus has yet been brought forward. See Klöcker and Schiönning (22).

In conclusion I should like to take this opportunity of stating that this work has been carried on in the Cambridge University Botanical Laboratory, by permission of Professor Marshall Ward, to whom my thanks are also due for his unfailing help and advice.

Note. Since the above was in print I have been able to see a copy of Steuber's paper (14) in the *Centralblatt für Bakteriologie, &c.*, Abth. II, Bd. VI, No. 7. The author describes four varieties of *S. anomalus*, which differ considerably with regard to the forms of the colonies on plate-cultures, temperature-limits for vegetative growth and spore-production, liquefaction of gelatine, and behaviour with various sugars. The 'variety I' approaches most nearly the form described above. It ferments dextrose, laevulose, and saccharose completely, but

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does not ferment maltose, lactose, and galactose, being able, however, to grow on solutions of the latter sugars. There is an abundant formation of ethyl acetate and acetic acid, together with butyric acid; but the author makes no mention of amyl acetate, which is produced abundantly by the variety which I have investigated. It liquefies gelatine comparatively rapidly, and the maximum temperature for film-formation is rather higher than that for my variety, viz. 37° – 40° C. Judging from the description, the forms of the colonies on 10% beer-wort gelatine resemble those shown in Fig. 2.

BIBLIOGRAPHY.

1. HANSEN, E. CHR.: Sur la germination des spores chez les *Saccharomycètes*. Comptes-rendus des travaux du laborat. de Carlsberg, vol. iii, livr. 1. Kopenhagen, 1891.
2. LINDNER, P.: Wochenschrift für Brauerei. 1892, p. 75.
3. JÖRGENSEN, A.: Die Microorganismen der Gährungsindustrie.
4. LUDWIG, F.: Lehrbuch der niederen Kryptogamen.
5. FISCHER, B., and BREBECK, C.: Zur Morphologie, Biologie und Systematik der Kahmpilze, der *Monilia candida*, Hansen, und des Soorerregers. Jena, 1894.
6. KLÖCKER, ALB.: Recherches sur les *Saccharomyces Marxianus*, *S. apiculatus* et *S. anomalous*. Comptes-rendus des travaux du laborat. de Carlsberg, vol. iv, livr. 1. Kopenhagen, 1895.
7. NIELSEN, J. CHR.: Sur le développement des spores du *Saccharomyces membranaefaciens*, du *S. Ludwigii* et du *S. anomalous*. Comptes-rendus des travaux du laborat. de Carlsberg, vol. iii, livr. 3.
8. HANSEN, E. CHR.: Sur la vitalité des ferments alcooliques et leur variation dans les milieux nutritifs et à l'état sec. Comptes-rendus des travaux du laborat. de Carlsberg, vol. iv, livr. 3. Kopenhagen, 1898.
9. WEHMER, C.: Ueber die Verflüssigung der Gelatine durch Pilze. Chemiker-Zeitung, 1895, No. 91, 2088.
10. SCHUKOW, I. VON: Gär- und Konkurrenzversuche mit verschiedenen Hefen. Centr. für Bakt., &c., 2. Abth., Bd. ii, 1896, p. 359.
11. KLÖCKER, ALB., and SCHIÖNNING, H.: Centr. für Bakt., &c., 2. Abth., Bd. ii, 1895, p. 777.
12. SHIEWECK, D.: Biol. Centr., 1898, No. 27, pp. 140, 141.
13. YABÉ, K.: Bulletin Impérial, Univ. Tokio. College of Agriculture, vol. iiii, 1897, No. 3, p. 221.
14. STEUBER, L.: Zeitschrift für das ges. Brauwesen, 1900, No. 1-3.
15. KUJAWSKI, K. VON: Ibid., 1900, p. 111.
16. HANSEN, E. CHR.: Kritische Untersuchungen über einige von Ludwig und Brefeld beschriebene Oidium- und Hefenformen. Bot. Zeit., 1892, No. 19, p. 312.
17. LUDWIG, F.: Berichte der deut. Bot. Gesellschaft, Bd. iv, Heft 11.
18. BREFFELD, O.: Untersuchungen aus dem Gesamtgebiete der Mykologie, Heft ix. Die Hemiasci u. die Ascomyceten, pp. 124-143, 1891.
19. HANSEN, E. CHR.: Centr. für Bakt. u. Parasitenkunde, Bd. v, 1889, p. 632 ff.
20. REESS, M.: Bot. Untersuchungen über die Alkoholgährungspilze. Leipzig, 1870.
21. BEYERINCK: Centr. f. Bakt., Bd. xi, 1892, p. 68.
22. KLÖCKER and SCHIÖNNING. Extrait du Résumé du Compte-rendu des Travaux du Lab. de Carlsberg, vol. iv, 1896, p. 63; and Centr. f. Bakt., 2. Abth., Bd. ii, 1896, p. 185.
23. SMITH, THEOBALD: Centr. f. Bakt. u. Parasitenkunde, Bd. vii, pp. 225 and 502.

EXPLANATION OF FIGURES IN PLATE XIII.

Illustrating Mr. Barker's paper on a *Mycoderma* Yeast.

Fig. 1. Photograph of colonies on a plate of laevulose-Mayer gelatine (nat. size). Darker colonies becoming moist through absorption of liquefied gelatine. Two months old. At ordinary temperatures.

Fig. 2. Photograph of colonies on a plate of beer-wort gelatine (nat. size). Two months old. At ordinary temperatures.

Fig. 3. Plate-colonies on beer-wort gelatine at ordinary temperatures. The gelatine had partially liquefied. A, B, C, D, E, F, successive stages as shown on a seven days' plate. $\times 2$.

Fig. 4. Colony on beer-wort gelatine plate. Gelatine solid. One month old. At ordinary temperatures. $\times 4$.

Fig. 5. Beer-wort gelatine plate-colony at ordinary temperatures, showing mycelium formation. Two months old. $\times 4$.

Fig. 6. Colonies on saccharose-gelatine plate at ordinary temperatures. Three months old. A. Dot-like colony. B. Colony with partial mycelial development. C. Colony with well-developed mycelium. $\times 6$.

Fig. 7. Plate-colony on laevulose-Mayer gelatine at ordinary temperatures. One month old. A. As seen from above. B. Side view. $\times 6$.

Fig. 8. Fatty-looking plate-colony on laevulose-Mayer gelatine at ordinary temperatures. One month old. $\times 4$.

Fig. 9. Successive stages of cell-formation by budding from a single cell. A, at 1.45 p.m. B, at 2.55 p.m. C, at 4.20 p.m. D, at 5.30 p.m. E, at 7.30 p.m. From hanging-drop culture in beer-wort gelatine. Gelatine partially liquefied, thus accounting for changes in position of the cells. At 18.5°–19°C. $\times 1000$.

Fig. 10. Vegetation of secondary films (veils). A, on beer-wort. B, on saccharose-Mayer solution. C, on laevulose-Mayer solution. $\times 480$.

Fig. 11. Stages in the germination of spores at 15°C. Group of four spores. A, at 3 p.m. B, at 9.45 a.m., three days later. C, at 11.15 a.m. D, at 2 p.m. E, at 4.35 p.m. F, at 9.30 p.m. Single spore: G, at 3 p.m. H, at 9.30 a.m., four days later. I, at 1.15 p.m. $\times 1000$.

Fig. 12. Portion of mycelium developed at edge of plate-colony on beer-wort gelatine (see Fig. 5). A. Terminal filament. B. Older branched portion. $\times 480$.

Fig. 1.

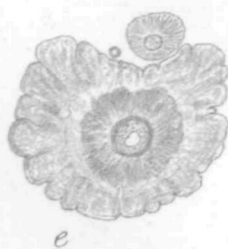
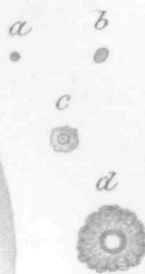
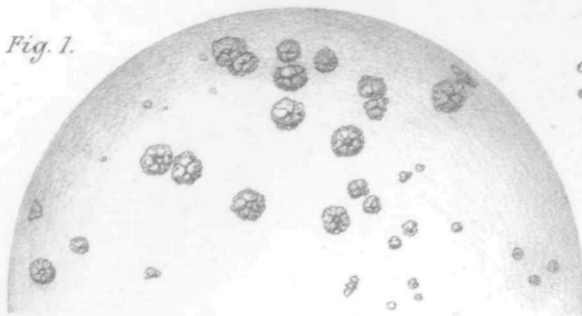


Fig. 3.

Fig. 2.

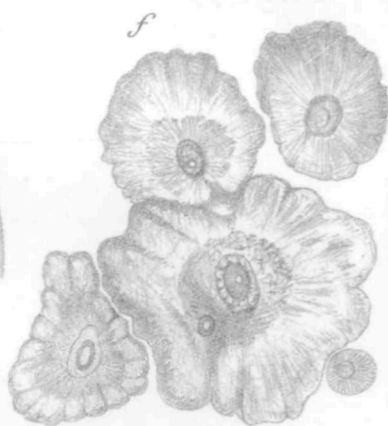
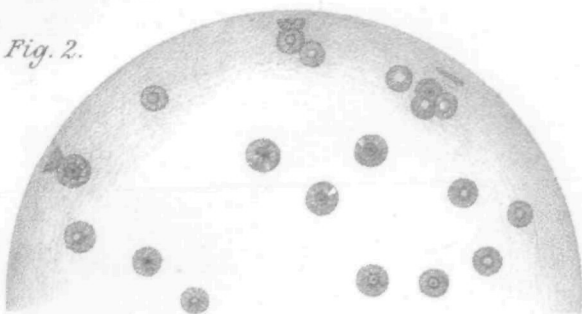
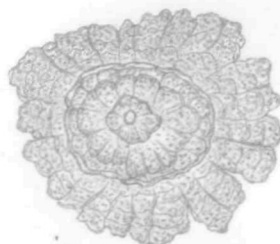
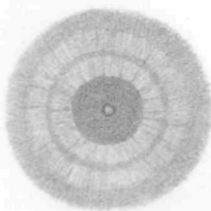


Fig. 4.



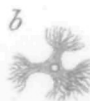
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Fig. 7.

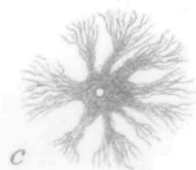


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Fig. 6.



b



c

Fig. 8.



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Fig. 1.

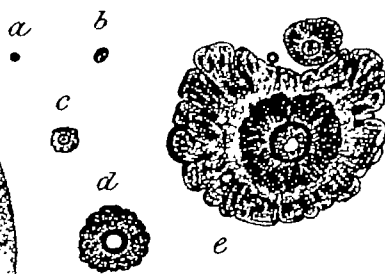
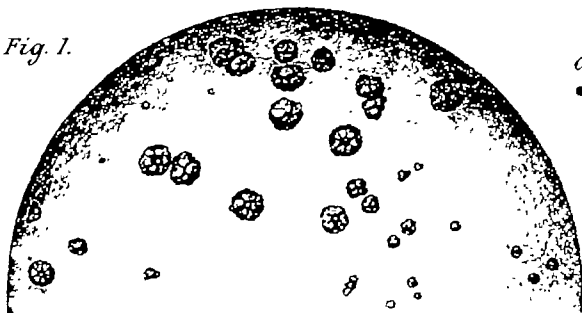


Fig. 3.

Fig. 2.

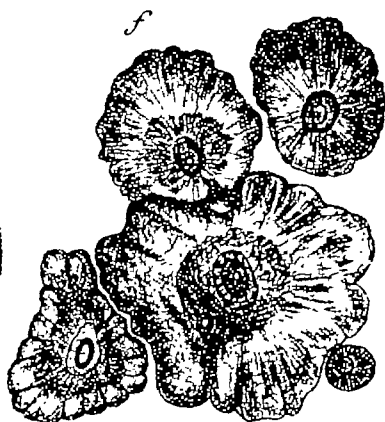
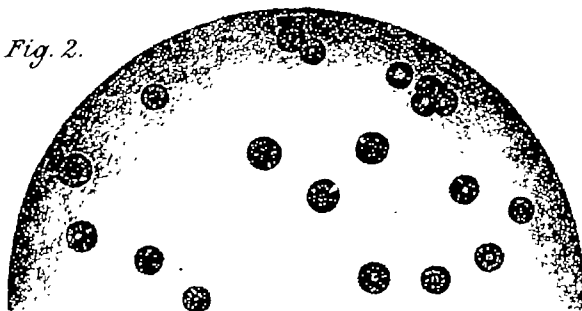


Fig. 4.

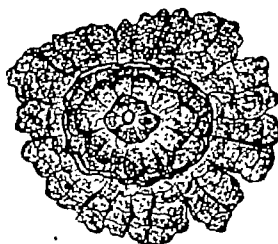
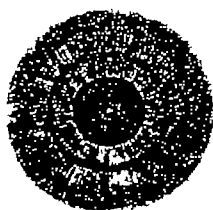
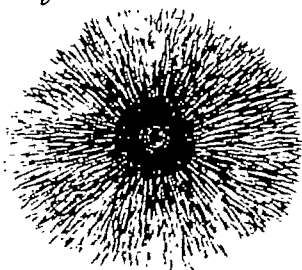


Fig. 5.



a

Fig. 7



Fig. 6.

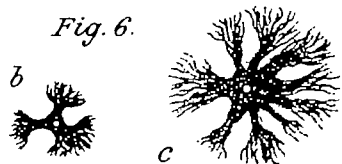


Fig. 8.



Barker del.

BARKER. — A FRAGRANT "MYCODERMA" YEAST.

