FACTORS AFFECTING THE MORPHOLOGY OF CANDIDA ALBICANS*
DAN OTHO McCLARY**

INTRODUCTION

Most morphological studies on the yeast-like fungi have been conducted on natural substances—malt extract, corn meal, and various vegetable decoctions such as potato and carrot, as broth or solidified with agar. Since growth on these chemically unknown substances yields a great variety of morphologically different forms, there is much controversy as to their true morphologies and the causes for their variations. For this particular group of fungi and perhaps for others which are somewhat more stable morphologically, more precise physiological information seems to be needed than can be obtained on natural media in order to arrive at definite conclusions concerning morphological variation. It is the purpose of this study to define the morphology of a well-known species grown in media of known chemical composition under carefully controlled physical condition, in the belief that much of the existing confusion in the taxonomy of this group of fungi can be eliminated by use of such an approach. Candida albicans was chosen because of its extreme variations in form, and because of the extensive studies which have been made upon it.

I am indebted to Dr. Carroll W. Dodge for suggesting this problem and for his generous help throughout the course of the study.

Classification.—Although the yeast-like organism described by Robin in 1847 as the cause of the disease known in modern literature as thrush, muguet, sapinho, and Soor has been known for over a hundred years, there is apparently little agreement among mycologists as to its taxonomic position or even its name. Robin (1853) first named the organism Oidium albicans. Quinquaud (1868), realizing that the organism did not belong in Oidium, placed it in his new genus, Syringospora, naming it Syringospora Robinii. He not only described the characteristic clusters of blastospores, but he also presented drawings definite enough for one to be reasonably certain that he referred to the organism now known as Candida albicans (Dodge, 1935; Skinner, 1947). In 1877 Grawitz called attention to the differences between the yeast form and the mycelial form. He also described chlamydozspores and even discussed the action of the media on morphology; but it is thought that he may have been working with mixed cultures because of his crude culture techniques. He believed this organism to be the same as Mycoderma vini. Reess, in 1877, showed that the organism was distinct from Mycoderma vini and called it Saccharomyces albicans. Plaut, in 1885, was the first to apply modern cultural technique. He identified the mycelial form with Monilia candida Bonorden on decaying wood. Stumpf, in 1885, concluded that he had two organisms, one

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**Assistant Professor of Microbiology, Southern Illinois University, Carbondale, Ill.

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filamentous and one yeast, both liquefying gelatin. In 1885 Baginsky studied the organism on various media, and Klemperer produced experimental mycosis from intravenous injections.

Audrey (1887) first proved the connection between yeast and mycelial forms, showing that yeast cells were more common on solid media, filaments in liquid. Roux and Linossier (1890) studied the physiology in considerable detail, giving extensive notes on carbon and nitrogen metabolism without definitely describing the biochemical reactions. They describe their organism as producing white, elevated, creamy colonies, with surface slightly furrowed on cooked carrot. At first the yeast cells predominate, then there are some filaments for a short period, and finally yeast cells again. On liquid media the filamentous forms predominate except in malt extract. On most fruits (except melon) and on peptone gelatin, the yeast form is abundant. On sucrose gelatin both forms are found. No ascospores were observed, but chlamydospores were not uncommon on most media used.

In 1923 Berkhout introduced the new genus, Candida, and designated Monilia candida Bonorden 1851 as the type species based on a culture isolated by Kloecker under the name Monilia candida but "evidently not that species" (Dodge, 1935). To avoid the use of a repeating binomial, Berkhout changed the name to Candida vulgaris.

In 1934 Diddens and Lodder adopted Berkhout's genus, Candida, but they designated another species, Candida albicans, as the type. This name has persisted in spite of the objections of most of the other well-known mycologists including Dodge (1935), Conant (1940), Mackinnon and Artagaveytia-Allende (1945), and Skinner (1947). All agree that it should be Syringospora Quinquaud 1868 by right of priority. This organism appears in the literature under a number of other names, but most of the important work concerning it may be found under Monilia, Syringospora, and Candida. The organism used in this study was received from the American Type Culture Collection as Candida albicans, and that name will be used in this paper.

General Morphology.—There are many morphological descriptions of this highly pleomorphic organism in the literature (Quinquaud, 1868; Grawitz, 1877; Audrey, 1887; and many others). The close correlation between the physiology and morphology of this organism is generally recognized, so that culture conditions and the medium used are always given with the morphological description. Skinner's description (1947) is a generally accepted one:

Except for the chlamydospores there is little other morphological detail that will set a strain of C. albicans apart from the other species. Freshly isolated cultures show little tendency to formation of true or pseudomycelium unless grown in starvation media below the surface, as in scratch corn meal agar plates or potato infusion broth, or in sugar-free beef peptone gelatin stabs. Grown on ordinary Sabouraud agar the cells are almost exclusively of the budding yeast type. Strands of mycelium may penetrate into the substrate after prolonged incubation, but they are much more numerous and appear more promptly along the scratch in corn meal agar. Blastospores are invariably produced from the strands, but the arrangement of blastospores varies so much between isolates that discussion of this
has little value in a review of this sort. They tend to occur in ball-like clusters in fresh isolates, but not to the extent that they do in Candida albicans var. stellatoidea.

**Morphological variation.**—Morphological variations described in the literature are of two distinct types:

1. Irreversible changes called "degeneration" (a seemingly gradual change) and "dissociation" (a sudden but irreversible change) involving a mutation.

2. Reversible changes depending entirely on environmental conditions.

**Irreversible changes.**—This type of variation has been studied by Negroni (1935), Mackinnon (1940), Mickle and Jones (1939), Cavallero (1939), Martin and Jones (1940), and Conant (1940). Mackinnon (1940) described "membranous variants" and "lethal variants." In the "membranous variants" the blastospores become elongated into filaments, causing a characteristic wrinkled, or in more advanced variants, a spiky hard colony surrounded by a filamentous halo. In liquid medium this variant produces a mucous veil and the virulence diminishes. The biochemical properties do not suffer qualitative changes. The "lethal variation" is characterized by a lower rate of growth, a great diminution or total loss of virulence, and by increasing difficulty to produce mycelial growth. These variations may occur spontaneously as described by Mackinnon, or they may be induced by toxic substances such as immune serum (Negroni, 1935) or by lithium chloride and immune serum (Mickle and Jones, 1939).

Although the existence of these "dissociations" are accepted by most mycologists, Langeron and Guerra (1939) concluded, after their investigations of these "irreversible variations" made over a period of some ten years, that the S (smooth phase) is the normal one and the R (rough phase) develops as a result of various factors, chief of which are the reaction (pH) of the medium and "elongation factors" (presence of carbon dioxide, nutrients of a high molecular weight, and nitrates). These variations were reversed when the organism was transferred to fresh media. They did not find irreversible variations as reported by Mackinnon and others.

**Reversible changes.**—It is with this type of variation which occurs promptly when the organism is transferred from one set of culture conditions to another that this study is primarily concerned. In 1930 Talice published perhaps the most complete study and review of the factors influencing the reversible changes in this organism. He determined that production of filaments depends upon partial anaerobiosis, weak concentrations of nutrients in the culture medium, the strain of the organism used, the treatment it has undergone, and the age of the culture. He believed that the filamentous form is always the young form; the yeast form is the old form.

In 1938 Langeron and Guerra found the formation of filaments to be stimulated by prolonged culture in the laboratory, presence of high concentrations of carbon dioxide, and changes in the constituents of the medium during the course of growth, particularly the change in pH. Morquer and Nystérikas (1948) reported that certain concentrations of heteroxine (beta-indole-acetic acid) stimulate filament formation.
Nickerson and Jillson (1948) found that a metabolic product of *Trichophyton rubrum* would inhibit the filamentous phase of *Candida albicans* but had no effect on the yeast phase. They considered that a separate enzyme system controlled each of these two phases and that the morphology of any given culture depended upon a stimulation or suppression of one or the other of these two systems which are supposedly competing for the same substrate. Nickerson (1950) again attributed the morphology of the yeast-like fungi to a delicate balance between growth and cell division. If the balance were upset in such a manner as to permit only growth to occur, elongated cells without cross walls would be formed. Cell division is, according to him, associated with the maintenance of intracellular sulfhydryl (-SH) groupings. In slide cultures of *Candida albicans* grown on a synthetic medium consisting of glucose, ammonium sulfate, inorganic salts, and biotin, Nickerson found only the yeast form. When commercial or purified starch was substituted for the glucose, abundant filamentation and chlamydomycoses were produced. By adding cysteine to the medium, filamentation and chlamydomycose formation were prevented and only yeast cells were formed. He concluded there must be a certain amount of a readily assimilable carbohydrate such as glucose in order to maintain the high oxidation-reduction potential essential for the intracellular -SH groupings required for proper cell division.

In general, one must conclude from the findings in the literature that where conditions are favorable for rapid multiplication, as with easily assimilable carbohydrates and with abundant aeration, the unicellular yeast forms predominate. Reduced oxygen tension, starvation media, liquid media in general, high pH, high temperature, or practically any condition or set of conditions which inhibits growth but does not stop it entirely, tend to produce the mycelial growth of *Candida albicans*.

General physiological characteristics.—In practically every taxonomic work dealing with this organism, its ability to produce acid and gas from various carbohydrates has been used. Most workers agree that the organism produces both acid and gas from glucose, fructose, mannose, and maltose; acid and sometimes gas from galactose; and acid but never gas from sucrose. Kluyver and Custers (1940) and van Niel and Cohen (1942) have published papers concerning the biochemistry of carbohydrate fermentation of the yeast-like organisms. According to van Niel and Cohen, there is no essential difference between the fermentation of glucose and sucrose by *Candida albicans* except that it occurs at a considerably faster rate in glucose.

In addition to carbohydrate fermentation tests, most authors also include a study of various nitrogen compounds as possible sources of assimilable nitrogen for the organism. Most of this work has generally been on synthetic media consisting of a sugar for a carbon source and inorganic salts (auxanograph). Wickerham (1946) showed that certain nitrogenous compounds which have been reported as unassimilable are readily used if the proper growth factor or
MC CLARY—Candida albicans

factors is present in sufficient quantity in the auxanograph medium. Burkholder (1943), using chemically defined media, found that biotin is required for the growth of Candida albicans and that thiamin is stimulating. The work of Morquer and Nystérakis and of Nickerson and his co-workers was done largely upon chemically defined media. However, except for the substitution of starch for sugar by Nickerson in one of his media, their work consisted of studying the various substances which are supposed to have certain physiological effects on cell elongation or cell division. There has apparently been no attempt to determine the effects of altering various other essential components of the medium.

Methods

The organism used for the greater part of this study was obtained from the American Type Culture Collection as Candida albicans 2094. Five other cultures were sent by Dr. J. E. Mackinnon at the request of Dr. Carroll W. Dodge. These organisms were maintained on media consisting of: glucose, 1 per cent; Difco yeast extract, .5 per cent; and agar, 2 per cent.

Culture media.—The medium used, essentially that described by Olson and Johnson (1949) and hence to be referred to as "basal medium," is as follows:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>10.0 gm.</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>1.0 gm.</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.2 gm.</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>10.0 mg.</td>
</tr>
<tr>
<td>Amm. citrate (dibasic)</td>
<td>6.0 gm.</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>50.0 mg.</td>
</tr>
<tr>
<td>L-asparagine</td>
<td>2.0 gm.</td>
</tr>
<tr>
<td>Biotin</td>
<td>2.5 microgm.</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>75.0 microgm.</td>
</tr>
<tr>
<td>Calcium pantothenate</td>
<td>1.0 mg.</td>
</tr>
<tr>
<td>Inositol</td>
<td>5.0 mg.</td>
</tr>
<tr>
<td>Thiamine-HCl</td>
<td>200.0 microgm.</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>200.0 microgm.</td>
</tr>
<tr>
<td>Zinc sulfate</td>
<td>400.0 microgm.</td>
</tr>
<tr>
<td>Ferric ammonium</td>
<td>250.0 microgm.</td>
</tr>
<tr>
<td>Copper sulfate</td>
<td>25.0 microgm.</td>
</tr>
<tr>
<td>Bacto agar</td>
<td>20.0 gm.</td>
</tr>
<tr>
<td>Distilled water to</td>
<td>1000.0 ml</td>
</tr>
</tbody>
</table>

To facilitate the preparation of the several media required, stock solutions of vitamins and trace elements were prepared, preserved with toluene, and stored in the refrigerator.

In addition to variations of the above medium, various natural media with modifications as indicated were used. These include Bacto yeast extract, Bacto peptone, Bacto malt extract, corn steepwater, Bacto beef, Bacto corn-meal agar, and potato and carrot decoctions. Various sugars (C.P.) and other chemicals were used as indicated.

Although most mycologists insist that morphological studies should be made in situ as cover-glass or slide cultures or that the cultures be examined directly on the petri plate (Skinner, 1947), this method has been used to only a limited extent in this study. Almost all this work was carried out on agar slants or broth cultures in test-tubes. This method was believed necessary for at least four reasons: (1) With the many hundreds of cultures used in such a study, so much time would be consumed in making microcultures that they could not very well be continuously observed. (2) Slide and petri-dish cultures, when subjected to prolonged examination, become much more easily contaminated. (3) It was found that, probably due to the rapid exhaustion of nutrient material, slide cultures did
not undergo all the changes which were observed on slants. (4) A macroscopic as well as a microscopic examination was desired for each culture. Petri-dish and cover-glass cultures were, therefore, used only to verify observations made on slant cultures.

Slides for microscopic examination were made by transferring a bit of material from the slant to a slide upon which had been previously placed a drop of water or staining solution. A nichrome wire hook bent at a right angle about 3/8 inch from the end was used rather than a loop because the cultures were, under certain conditions, so tough that they resisted any amount of pressure that could be applied to them with a loop. Acetocarmine was found to give excellent results, the organism staining a bright red against a relatively colorless background. Since this stain evaporates quite rapidly, it was necessary to seal the preparation soon after the cover slip was in place. Turtex slide-ringing cement obtained from the General Biological Supply House, Inc., Chicago, Illinois, was found to be very satisfactory for sealing.

**Verification of the Species**

For morphological verification, agar slants of basal medium, .5 per cent yeast extract, and .5 per cent malt extract were inoculated with *C. albicans* A.T.C.C. 2091 and incubated at room temperature (25–30° C.).

For biochemical verification two types of media were used: (1) .3 per cent peptone, 10 per cent gelatine in distilled water for gelatine liquefaction tests; and (2) a series of nine carbohydrate media consisting of .3 per cent peptone with brom thymol blue indicator, and sugars as follows: (1) glucose, (2) fructose, (3) mannose, (4) galactose, (5) maltose, (6) sucrose, (7) lactose, (8) trehalose, (9) no addition.

The gelatine was dispensed in "18 × 150 mm." test-tubes; the carbohydrate media in large Smith fermentation tubes, and all were autoclaved at 12 pounds pressure for 15 minutes. After cooling, tubes of gelatine were inoculated in duplicate, using the stab method, and incubated at 25° C. The fermentation tubes were inoculated in duplicate with a small amount of culture taken with a hook from an agar slant and were incubated at 37° C. for five days.

In addition to the above fermentation tests, Durham fermentation tubes were prepared, using "23 × 185 mm." test-tube with a "10 × 75 mm." test-tube for a gas vial. A fermentation medium consisting of 3 per cent peptone in distilled water with brom thymol blue indicator was divided into three parts and 5 per cent quantities of the following sugars were used in each respectively: (1) glucose, (2) sucrose, and (3) galactose. The fermentation tubes were inoculated very heavily—each one being inoculated with approximately all of a slant culture which had been grown previously, using a corresponding sugar as a carbon source. It was hoped that the great number of cells initially present would provide anaerobic conditions.
Results.—Microscopic examinations of slides prepared from bits of the cultures obtained from near the center of the slants revealed, in all cases, a complex mixture of filaments with verticels of blastospores and budding yeast cells. After several days, there were also observed numerous thick-walled, round chlamydo- spores appearing terminally on thick filaments and free in the medium. The results of the fermentation tests with a light and a heavy inoculation used in this study are given in tables I and II, respectively.

**TABLE I**

ACID AND GAS PRODUCTION BY _CANDIDA ALBICANS_ A.T.C.C. 2091 IN PEPTONE CARBOHYDRATE MEDIA. LIGHT INOCULUM.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Reaction</th>
<th>Gas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Acid</td>
<td>+*</td>
</tr>
<tr>
<td>Fructose</td>
<td>Acid</td>
<td>+</td>
</tr>
<tr>
<td>Mannose</td>
<td>Acid</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>Acid</td>
<td></td>
</tr>
<tr>
<td>Maltose</td>
<td>Acid</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Acid</td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>Strongly alkaline</td>
<td></td>
</tr>
<tr>
<td>Trehalose</td>
<td>Acid</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Strongly alkaline</td>
<td></td>
</tr>
</tbody>
</table>

*+, gas produced; —, no gas produced.

**TABLE II**

ACID AND GAS PRODUCTION BY _CANDIDA ALBICANS_ A.T.C.C. 2091 IN PEPTONE CARBOHYDRATE MEDIA. VERY HEAVY INOCULUM.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Reaction</th>
<th>Gas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Acid</td>
<td>+*</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Acid</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>Acid</td>
<td>+</td>
</tr>
</tbody>
</table>

*+, gas produced; —, no gas produced.

It is noted (Table I) that glucose, fructose, mannose, and maltose are readily fermented with acid and gas. It is also observed that, although galactose, sucrose, and trehalose are utilized, producing an acid and a rich growth, gas is produced in galactose (Table II) only under conditions unsuitable for further growth and in sucrose not at all. These observations will be again referred to in the discussion of nutrition and its relation to morphology. Finally, this organism brought about a complete liquefaction of the nutrient gelatin, a characteristic included by most taxonomists.
These results are thus in agreement with the taxonomic requirements presented in the literature and reviewed by Skinner (1947). According to Skinner, the most obvious morphological characteristic of this species is the production of round (or nearly so) heavy-walled terminal cells called chlamydospores. Strands of mycelium may or may not develop from which blastospores or buds are invariably produced. It is also generally agreed among mycologists that Candida albicans is the only species of the yeast-like fungi which ferments (produces gas) glucose, galactose, and maltose, but not sucrose and lactose.

Since all of the above characteristics are possessed by Candida albicans A.T.C.C. 2091, it is concluded that it is as typical a culture as can be obtained and is a suitable one for this type of study.

**Influence of Hydrogen Ion Concentration**

In this experiment, the basal medium was adjusted in series at pH 3 through 9 by means of a Beckman pH meter with approximately 5 per cent HCl and 5 per cent NaOH. The media were then dispensed in test-tubes suitable for slants and were autoclaved at 12–15 pounds pressure for 12 minutes. In addition to this medium, Bacto yeast extract agar, corn steepwater agar, and Bacto malt-extract agar were prepared as above at pH values of 5 and 8. All media except that adjusted to pH 3 were inoculated as slants. The medium of pH 3 would not solidify after autoclaving and was inoculated by the stab method. These cultures were incubated at 24°C for two days.

*Results.*—In general, there was little difference in growth of the organism in the ranges of pH 4 through 7. Growth was poor at pH 3 and 8, and no growth was noticeable on the pH 9 culture for several days. When growth did occur on this medium, it began as a little colony at the very thin part of the slant and gradually spread down over the thicker portion. When this colony was used to inoculate tubes of the same medium, growth occurred promptly.

On the basal medium at extreme pH ranges (3, 8, and 9), microscopic examination revealed a preponderance of yeast-like cells and large, spherical, thick-walled chlamydospores. The filaments that were present were of irregular shape and had a swollen appearance (pl. 15, fig. 1). The most filamentous growth occurred on the basal medium at pH 5. At ranges of pH 4 and 6, the filaments were not so regularly thread-like as those grown at pH 5, but they, like it, did not develop chlamydospores within 2 days. Although the culture grown at pH 7 was quite filamentous, it consisted of more yeast-like cells and irregular filaments than did those grown at a slightly lower pH. Chlamydospores were also numerous. Cultures on malt extract at corresponding pH ranges had very much the same morphology as those grown on basal medium. Yeast extract and corn steepwater produced a preponderance of yeast cells under all conditions.

1 The effect of autoclaving was determined on these media and some change was observed. These changes were never over .5 of a pH unit, however, and always occurred in the direction of neutrality.
Influence of Nutrients

In order to determine the basic nutritional requirements of this organism, a series of media was prepared with each medium lacking a different ingredient of the complete basal medium. These media were prepared as slants, and each was inoculated from a stock culture maintained on glucose peptone agar. Slants of the complete basal medium were inoculated for controls. All were incubated at 24°C.

After one day's growth the slants were examined macroscopically; then bits of material taken from them with a nichrome wire hook were mounted on slides, stained, and examined microscopically. Examinations were made after two days, three days, and longer periods to determine the effect of prolonged incubation.

Results.—Macroscopic examination of the day-old cultures revealed considerable difference, not only in the amounts of growth on the various media but also in their gross morphologies. Poor, though distinct, growth was observed on media which were lacking in all vitamins, biotin alone, phosphorus, potassium, and sugar. All the other media except that lacking calcium pantothenate, which was so little different as to be doubtful, gave almost identically luxuriant growth.

The gross morphologies of the cultures resulting on these media were quite as distinctly different as the growth quantities. There was little difference in the growth resulting from lack of sugar, biotin or all vitamins, and phosphate, each being almost pure white, very soft, and creamy. The growth resulting on a potassium-deficient medium, though not so distinctly differing from the above in young culture, became rather dry and granular with a yellow-green color. Samples of each of the above cultures could be very easily removed with a wire loop. The growth resulting on the rest of the media was a pale olive-buff or nearly white with a velvety appearance. These cultures were found to consist of a distinct, tough membranous mat covering the surface of the agar. It was necessary to use a wire hook to tear pieces of this membrane from the slant. With little difficulty the entire membrane could be removed intact.

When samples from the above cultures were examined microscopically, it was observed that the organism had responded to each nutritional deficiency with a distinctly different morphology. As one would expect from the lack of response to any of the vitamins except biotin, omitting biotin alone had the same effect as omitting all the vitamins. The growth on each medium consisted essentially of oval yeast cells with occasional, rather short, thick mycelial strands (pl. 15, fig. 2). The effect of the lack of sugar could not be differentiated from that obtained on a biotin-deficient medium. In this medium the only carbon source was ammonium citrate which, for this organism, is a very poor one.

Material from the phosphate-deficient medium consisted of very long mycelial strands with comparatively few typical yeast cells and blastospores. The most conspicuous characteristics were the numerous chlamydospores which developed in a very short time and the large vacuoles in the hyphae and yeast cells (pl. 15, fig. 3).

Jones and Peck (1940) have reported a green pigment produced by Candida albicans and C. stellatoidea.
The potassium-deficient medium also yielded a growth form of a rather distinct morphology. Although there were practically no free yeast cells, neither was there ever a true mycelium. The entire growth consisted of clusters or rosettes of pseudohyphae composed of elongated, distinctly separate cells (pl. 15, fig. 4).

The samples from all the rest of the media were found to be just as much alike microscopically as they were macroscopically. All consisted of very dense entanglements of very long, thread-like, apparently non-septate hyphae. The cultures contained very few yeast cells and blastospores when young, but as they grew older these forms began to predominate (pl. 15, fig. 5). The effect of age will be discussed in more detail in a later section.

As indicated above, the basal medium contains several constituents which are not necessary for good growth. To test these effects further, the following medium was prepared in slants and inoculated:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>10.0 gm.</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>10.0 mg.</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>10.0 mg.</td>
</tr>
<tr>
<td>Ammonium citrate (dibasic)</td>
<td>6.0 gm.</td>
</tr>
<tr>
<td>Biotin</td>
<td>2.5 microgm.</td>
</tr>
<tr>
<td>Calcium pantothenate</td>
<td>1.0 mg.</td>
</tr>
<tr>
<td>Copper sulfate</td>
<td>25.0 microgm.</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0 gm.</td>
</tr>
<tr>
<td>Distilled water to</td>
<td>1000.0 ml.</td>
</tr>
</tbody>
</table>

Growth on the above medium after two days was not as heavy as that obtained on the complete basal medium, though the morphology was the same. Since it was desired to obtain the best growth possible, a basal medium was prepared, consisting of all of the heretofore-mentioned substances, except the vitamins, riboflavin, calcium pantothenate, inositol, thiamine, and pyridoxine, and asparagin.

From the data obtained on the containers of the chemicals used, it was calculated that at least the following quantities of inorganic constituents were present per liter of medium under all conditions:

<table>
<thead>
<tr>
<th>Element</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium, less than</td>
<td>0.05 mg.</td>
</tr>
<tr>
<td>Phosphorus as phosphate, less than</td>
<td>0.02 mg.</td>
</tr>
<tr>
<td>Zinc,</td>
<td>0.0075 microgm.</td>
</tr>
<tr>
<td>Iron,</td>
<td>0.065 mg.</td>
</tr>
<tr>
<td>Copper,</td>
<td>0.0075 microgm.</td>
</tr>
<tr>
<td>Other sources of trace substances are from the distilled water and the agar.</td>
<td></td>
</tr>
</tbody>
</table>

Undoubtedly, most of these elements, especially magnesium, iron, and phosphorus, are required by this organism, but with the exception of phosphate, these requirements are so low that a demonstration of them is rather difficult. For information concerning the purification of media and the effects of various metallic ions on the growth and metabolism of fungi, the reader is referred to Perlman (1949).

Effects of various carbon sources.—The medium used was the basal medium previously described but with the omission of all of the vitamins except biotin, and the substitution of other possible carbon sources for sucrose. Large Durham fermentation tubes (25 ml. of medium) of the media were prepared, using 5 per cent sugars, and inoculated heavily from a culture previously grown on the complete basal medium. Since the only difference in any of the media was the carbohydrate, the complete medium is designated only by the name of the sugar.
The fermentation was at room temperature (25–30° C.). The results are given in Table III.

TABLE III

ACID AND GAS PRODUCTION BY CANDIDA ALBICANS A.T.C.C. 2091 IN SYNTHETIC CARBOHYDRATE MEDIA

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Reaction</th>
<th>Gas</th>
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<tr>
<td>Glucose</td>
<td>Acid</td>
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<tr>
<td>Fructose</td>
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<tr>
<td>Mannose</td>
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<tr>
<td>Galactose</td>
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</tr>
<tr>
<td>Sucrose</td>
<td>Acid</td>
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*Relative rates of gas production are indicated by the number of plus signs; — indicates no gas production.

In addition to fermentation tests, agar slants of the same basal medium were prepared using the above sugars as well as alcohol, glycerol, starch, and succinic acid as sole sources of carbon and in combination with each other. Various quantities of each compound were used to determine the effect of concentration. These media were inoculated and incubated at 24° C. for 24 hours.

Results.—Fermentation (gas production) did not become apparent in glucose medium until after two days and not in the others until several hours later. By the end of three days, both the glucose and mannose fermentations were quite active with considerable gas production. There was a small amount of gas in the fructose fermentation tube, but in the galactose, maltose, and sucrose tubes, there was still no activity. Eventually, the maltose fermentation was active and still later the galactose, but there was no gas production from sucrose after a month.

On slants containing the above medium, with 2 per cent concentrations of glucose, fructose, and mannose respectively, there was no noticeable macroscopic difference in growth. All cultures grown on these media, after one day, were soft, creamy white, and very easily removed from the slant with a wire loop. Microscopic examinations of all three of these cultures revealed a complex mixture of yeast cells and filaments (pl. 15, fig. 6).

The cultures grown on galactose, maltose, and sucrose media were almost white, rather dry, and so tough that a wire hook was required to tear portions from the slants. The growth on the galactose culture appeared somewhat more luxuriant than that obtained on the other media, and after a few days it became quite pubescent and yellow in color. Microscopically, the growth on galactose medium was the most purely mycelial of any of the cultures obtained on these three media, although all three were composed predominantly of long, thread-like, non-septate filaments (pl. 17, fig. 18).
Lactose and starch were not utilized by this organism, and the resulting growth was like that obtained on medium containing only citrate as a carbon source. The reactions of these media became alkaline.

Alcohol, glycerol, and succinic acid were utilized but the growth was almost entirely yeast-like (pl. 15, fig. 2). Two per cent succinic acid produced the richest growth of the three. These media became alkaline in reaction.

In all cultures in which 1 per cent alcohol was added to the sugar media, the growth became more filamentous than that obtained on sugar media alone. When 3 per cent alcohol was added, however, the resulting growth was characterized by rather large yeast cells in clumps and chains, with very frequent, rather short, pseudohyphae attached, presenting many clavate structures (pl. 16, fig. 7). When 5 per cent sugar concentrations were used, there was greater tendency in all media to the yeast-like phase. Growth resulting on galactose medium was still the most mycelial of the group.

All the above cultures were examined from time to time both macroscopically and microscopically to determine the effect of age. Microscopic examination never revealed a culture which became more filamentous with age. However, to the naked eye some of the cultures, particularly those which on the first day were practically pure mycelial structures, became more hirsute after four or five days longer incubation. Those apparently thread-like strands seen by the unaided eye extending out from the edge of the colony were each composed of a single long filament very thickly covered with dense clusters of blastospores. The central filaments were too small to be seen macroscopically, but there is little doubt that they had been produced long before they could be noticed. Microscopically, any sample taken from a culture, which had been almost entirely mycelial when only one or two days old, after several days revealed a preponderance of yeast-like cells. The mycelium which was still present was apparently devoid of protoplasm since it would not stain except for occasional granular structures. It was observed that the yeast cells developed between the membranous mat produced by the mycelium and the agar, and eventually broke through to the surface as the membrane degenerated. A culture which was yeast-like at the beginning was never observed to become more filamentous with age.

Influence of Temperature

Media.—The medium used in determining the effect of temperature upon filamentation was mostly the original basal medium, but almost every other medium used in this study was tested at various temperatures at one time or another. Agar slants were inoculated in triplicate, and one of each was incubated at 24° C., 37° C., and 40° C., and examined at 24- and 48-hour periods.

Results.—On media which were previously found to produce the yeast form at 25°–30° C., there was little difference in either the macroscopic or the microscopic appearance resulting from the three incubation temperatures. In the media which had been previously found to favor a mycelial form at room temperature
there was considerable difference. Grown at 24° C., these cultures were tough and membranous; at 37° C. and 40° C., they were no longer tough and membranous but soft and creamy. Microscopic examination revealed in those cultures incubated at 24° C. very thin, thread-like filaments with few yeast cells and blastospores (pl. 16, fig. 8), and in those incubated at 37° C. and 40° C., thick, septate pseudomycelium and rather large yeast cells in rosette-like clusters (pl. 16, fig. 9). Their general appearance is much more yeast-like than those incubated at lower temperatures.

**Influence of the Consistence of the Medium**

*Methods.*—A series of liquid media was prepared as previously described but with each medium lacking one essential nutrient. Tubes of these media and of the complete medium were inoculated in triplicate, and one of each was incubated at 24° C., 37° C., and 40° C. After two days' growth, the cultures were examined macroscopically, then they were shaken to provide uniform sampling. Samples were taken with a long, dropper-type pipette and mounted on slides for microscopic examination.

*Results.*—Heaviest growth occurred in the complete basal medium at all temperatures. There was considerable turbidity in the upper part of the medium and a flocculent sediment at the bottom. In the biotin-deficient medium there was a flocculent mass at the bottom of the tube and no turbidity in the upper part. In cultures from which sugar was omitted and also in those from which phosphate was omitted, there was a flocculent mass which settled rapidly when the tubes were shaken. Cultures lacking potassium were very granular and settled rapidly after they were shaken. Temperature had no visible effect upon gross morphology.

Microscopically, the differences which were obtained on different liquid media at different temperatures were not so distinct as they were found to be on solid media. This was especially true of the cultures on biotin- and sugar-deficient media, which were much more mycelial than corresponding agar slant cultures. The potassium- and phosphate-deficient media produced forms like those produced on agar slants of these media. The pseudomycelium consisting of rosettes of yeast-like cells were equally noticeable in the potassium-deficient medium. The other cultures consisted of long, thread-like filaments with numerous blastospores and yeast-cells.

There was little noticeable difference in cultures incubated at 37° and 40° C. Although high temperature inhibited filamentation on the slant cultures, this factor had surprisingly little effect on liquid media. The filaments became, perhaps, a bit thicker with more of a pseudomycelial tendency.

Tubes of the above media were also inoculated and aerated for two days by bubbling air through them. These were little different from those described for the non-aerated cultures above.
In general, most of the differences noted on agar slants were present in liquid cultures but they were less distinct. The pure mycelium and yeast forms obtained on agar slants were not obtained in liquid media.

Effect of anaerobism.—Mycologists generally agree that filaments are produced as a result of reduced oxygen tension, this conclusion having been reached mainly through comparison of growth in liquid and solid media. Since filaments were found more common in liquid media than on solid, they consider the difference to be due to the difference in oxygen available to the organism under the two conditions. Wickerham and Rettger (1939), however, described the growth of Candida albicans on corn meal agar under what they considered reduced oxygen tension, being accomplished by placing a cover glass over a developing colony on a petri dish or on a slide covered with a thin layer of agar. Langeron and Talice (1932) found that carbon dioxide had a stimulating effect on mycelium production. In order to test further the effect of anaerobic conditions, the following experiment was performed.

The chemical reservoir of a large desiccator (21-liter capacity) was filled with 10 per cent sodium hydroxide solution. Two petri plates and two agar slants containing sucrose basal medium were inoculated by heavy streaking from a similar culture. Approximately 150 gms. of pyrogallic acid were mixed with the sodium hydroxide solution. The cultures and a lighted candle were then introduced into the desiccator and the lid replaced. To insure sealing, the lid and rim of the desiccator were well greased with stopcock grease. For controls, like cultures were prepared and incubated outside the desiccator. All were incubated at room temperature for two days before examination.

Results.—The rich growth of the cultures incubated outside the desiccator and the almost complete lack of growth of those incubated inside the desiccator indicated that anaerobic conditions had been achieved. When material from all cultures was examined microscopically, there was little detectable difference. In both cases there were long filaments mixed with blastospores and yeast cells. The anaerobic cultures contained rather large vacuoles. When the cultures that had been incubated anaerobically were placed under aerobic conditions, they soon developed abundantly. Although all the plates had been uniformly streaked over a rather large area, most of the growth was at the edges, so that a thick widening ring was formed around the outside (pl. 16, fig. 10). A halo of hyphae surrounded the outer edge of the ring, but there were few within the surrounded area. This phenomenon is undoubtedly the same as that described and photographed by Magni (1948) in his work on reciprocal inhibition of pseudomycelium formation in parallel colonies. He believed that the lack of pseudomycelial development between parallel colonies was due to the lack of nutrients.

Effects of Various Other Substances

Various factors, in addition to those just discussed, have been reported to influence the morphology of this organism. Negroni (1935) reported the influence
of phenol in producing a rough (R) type colony of *Candida albicans* approximating the R type colony of bacteria. Mickle and Jones (1939) studied the effect of lithium chloride and immune serum on dissociation. Nickerson and Jillson (1948) found that the mycelial phase of *Candida albicans* was completely inhibited by culture filtrates of *Trichophyton rubrum*. Varying concentrations of beta indole acetic acid were found by Morquer and Nystérakis (1948) to be very influential in bringing about a filamentous form. Langeron and Guerra (1939) reported the influence of so-called "elongation factors" chief of which are high concentration of carbon dioxide and substances of high molecular weight such as peptone, and nitrates. Nickerson (1950) noted an inhibiting effect of cobaltous nitrate and proflavine on cell division in *C. albicans* with the consequent production of the mycelial form. According to him, .001 M cysteine not only inhibits chlamydomspore and mycelium formation (which he considers are brought about by the same factors) in his basal medium, but also counteracts the effect of cobaltous nitrate and proflavine. Most authors believe that a high carbon-low nitrogen ratio is also conducive to mycelium production.

Certain of the experiments were repeated in this study with varying degrees of success as will be indicated.

Methods.—The medium used was usually that described above, but peptone and yeast extract agar were sometimes used. Sucrose, glucose, and galactose were used as carbon sources. All of this particular phase of work was done on agar slants.

Effect of phenol.—Galactose basal media containing approximately .05 per cent and .1 per cent phenol were inoculated with *C. albicans* and incubated at 24° C. for 24 hours. Samples were taken from the slant and prepared as previously described for microscopic examination.

Results.—Macroscopically, both the above cultures were rather rough, somewhat granular, and soon became brown in color. Microscopically, these cultures were observed to consist of very thick, irregular pseudohyphae and large yeast cells. No chlamydomspores were observed (pl. 16, fig. 11).

Effects of cobaltous nitrate and cysteine.—The following media were inoculated with *C. albicans* and incubated at 24° C.:

1. Basal medium less all vitamins except biotin; 2 per cent galactose; .05 per cent cobaltous nitrate.
2. Medium as above except MgSO₄ was increased five fold.
3. Medium like No. 1; 2 per cent sucrose; .001 M cysteine.
4. Medium as above; 2 per cent sucrose; .001 M cysteine; .05 per cent cobaltous nitrate.
5. Medium as above; 2 per cent sucrose; .002 M cysteine.
6. Medium as above; 2 per cent succinic acid; .05 per cent cobaltous nitrate.
7. Yeast extract, 1 per cent; sucrose, 2 per cent; K₂HPO₄, .02 per cent; Co(NO₃)₂, .05 per cent.
8. Yeast extract, 1 per cent; sucrose, 2 per cent; K₂HPO₄, .02 per cent; Co(NO₃)₂, .1 per cent.
9. Peptone, 3 per cent; galactose, 2 per cent; K₂HPO₄, .02 per cent; Co(NO₃)₂, .05 per cent.
10. Peptone, 3 per cent; glucose, 2 per cent; K₂HPO₄, .02 per cent; Co(NO₃)₂, .05 per cent.

Results.—The above cultures were examined at the end of twenty-four hours and from time to time thereafter. In the 24-hour cultures, there were little
macroscopic or microscopic differences between Nos. 1, 2, 3, and 5 or the basal media, using the corresponding carbon sources with the cobaltous nitrate and cysteine omitted. The basal media containing sucrose and galactose as carbon sources produced a very mycelial form when cobaltous nitrate was present. Somewhat later those containing cobaltous nitrate became rough, rather granular and dry. Culture No. 2, with a high content of magnesium sulfate, remained more like the cultures previously described on basal medium. Culture No. 6, using succinic acid as a carbon source, was composed almost entirely of yeast cells, and no difference could be detected due to the addition of cobalt. Cultures 7, 8, 9, and 10 were much alike but greatly different from cultures grown on basal medium or on yeast extract or peptone media not containing cobaltous nitrate. In these natural media, cobaltous nitrate showed a definite growth inhibition not noted on the synthetic media. Growth developed very slowly on these media, beginning in small granular, brownish colonies on the thin part of the slant and slowly spreading down until, after several days, the whole slant was covered. Microscopically, these cultures were observed as clumps and chains of yeast-like cells. These last four media where cobalt nitrate was omitted gave complicated mixtures of mycelium and yeast cells.

The addition of cysteine seemed to have a slight toxic effect in the concentrations used, but it did not entirely prevent filaments from forming. When cysteine and cobalt nitrate were used, the organism became more yeast-like than when either was used alone, but this would seem to be due simply to the increased concentration of toxic substances. Chlamydospores were soon observed in these cultures when one or the other or both of these compounds was added to the media. At the concentrations used in these experiments, cysteine and cobalt had little morphological effect except for slight toxicity as indicated by the roughness of the cultures and decreased growth in certain cases.

Effects of other chemical substances.—No detailed studies were made on the other supposedly influential factors previously listed. The increased concentration of peptone to 5 per cent increased mycelial production as reported by Langeron and Guerra. Substances of high molecular weight were not tried as causes for filament production, since they were not employed in the medium which gave an almost pure mycelium. The highest molecular-weight compound used in this medium, other than the sugar, was ammonium citrate, and it was found that ammonium chloride gave an equally good mycelium and approximately the same amount of growth. Since nitrates were not employed at all, it is concluded that a good mycelium can develop in their absence. As for the necessity of a high carbon-low nitrogen ratio for mycelium production, it was found that when the carbon source was raised to a higher concentration than 5 per cent, there was a great tendency toward the yeast form.

3 This supports the findings of Abelson and Aldous (1950) concerning the antagonism of cobalt and other bivalent ions toward magnesium metabolism. They found that nickel and cobalt were less toxic to a variety of microorganisms when the magnesium content of the medium was increased.
Chlorides.—Although the effect of chlorides was not studied extensively, the observations made in the course of this work seem to be worthy of a brief remark here and of further study in the future.

While studying the effect of potassium on morphology (KCl being the potassium source) it was observed that increased concentrations of this salt up to 10 per cent would produce a purer mycelium when glucose was used as the carbon source than would the medium containing the normal, comparatively low concentration. Since it had been previously observed that potassium was necessary for the formation of a mycelium, higher concentrations of this element were believed to account for the mycelial stimulation. However, when 10 per cent NaCl was employed, using the normal amount of KCl in a glucose basal medium, this mycelium-stimulating (or yeast- and blastospore-retarding) tendency was observed to be as strong as in the 10 per cent KCl medium.

Morphology on Various Natural Media

Skinner (1947) has listed a number of natural media employed by mycologists in their morphological studies, but he preferred Benham’s corn-meal agar as prepared by Bernhardt (1946) and Anderson’s corn meal infusion for inducing mycelium and chlamydomospore production. Wickerham and Rettger (1939) found corn-meal agar very suitable for true mycelium production. Talice (1930) preferred potato infusion or potato agar for inducing filamentation. Sabouraud agar (glucose peptone agar) has found wide use in morphological studies, giving cells almost exclusively of the budding yeast type. Sugar-free beef peptone gelatine stabs have also been reported useful. Diddens and Lodder (1934) employed a number of natural media, among which the most used were malt extract, wort, wort agar, glucose peptone agar, and milk.

Media.—Various natural media, including Bacto malt extract, corn steepwater, yeast extract, Bacto peptone, Bacto beef, Bacto corn-meal agar, and potato and carrot decoctions, were used alone and in combination with the previously used sugars. In addition, some of these natural substances were added to the complete basal medium. These media, prepared as slants, were inoculated with a 24-hour-old culture grown on yeast extract-glucose agar at 24° C.

Results.—The malt-extract culture was the most filamentous of the group, having long, thread-like filaments with numerous blastospores. The growth of this culture was also quite heavy. Corn steepwater and yeast extract cultures were predominantly yeast-like. When yeast extract was added to the complete basal medium, which ordinarily produces the mycelial form, a yeast-like form was produced. Peptone cultures were always complicated mixtures of filaments and yeast cells. All the above media yielded fair growth, but the addition of mineral salts and sugars usually increased the growth. The Bacto beef and corn-meal agar cultures were fairly filamentous. However, most of the filaments were rather short, and in young cultures were swollen at the ends. These cultures showed very poor growth even with sugars and potassium phosphate added. The growth


on potato and carrot agar was quite good, being of the soft, creamy type. Both these cultures contained many pseudohyphae and a preponderance of yeast cells. The addition of sucrose to these media improved the growth, but the morphology was virtually unaffected. There was little better growth, if any, in any of these media than that obtained on the basal medium. In most cases it was inferior.

Morphological Comparisons of the A.T.C.C. Strain 2091 With Other Cultures of C. albicans

Five cultures of Candida albicans were obtained from Dr. Mackinnon which were without data except for the initials and numbers used to designate the individual strains. These cultures were designated as 1. H.M. 493, 1. H.M. 805, 1. H.M. 806, 1. H.M. 679, and 1. H.M. 582. Agar slants of galactose basal medium from which all vitamins except biotin were omitted were inoculated with these strains and were incubated at 24° C. for 24-48 hours. The cultures were then examined macroscopically and microscopically.

The slant culture 1. H.M. 493 was almost pure white, rather soft, and wrinkled. Microscopically, it was quite mycelial, but the hyphae were rather thick and twisted, indicating that, although the growth was quite heavy, the medium was not altogether suitable for the best growth of this organism (pl. 17, fig. 13). Culture 679 was rough, cream-colored, and quite soft. Microscopic examination revealed a fairly good mycelial growth and many somewhat lance-shaped yeast cells. Growth was good (pl. 17, fig. 14). Culture 805 did not grow very well on this medium. The growth appeared rather dry, almost white, and was easily removed from the slant with a wire loop. Microscopically, it was observed to be a mixture of yeast cells and pseudohyphae (pl. 17, fig. 15). Slant culture 806 was a very heavy, almost white, velvety growth and so tough and membranous that a wire hook had to be used to remove material from the slant. As one would expect from such a membranous material, this culture was observed microscopically to be very mycelial. The individual cells present were very narrow and rather long (pl. 17, fig. 16). Culture 582 was of a very soft, creamy, glistening white material. Growth was very rich. Microscopically, this culture was seen to consist preponderantly of small yeast cells, but there were occasional long, thread-like hyphae (pl. 17, fig. 17). Although chlamydoconidia are not shown in the photograph, they were later observed to occur frequently in chains of six or seven as well as individually at the tips of filaments.

The six strains of C. albicans, including the five Mackinnon strains and the A.T.C.C. strain 2091, differ quite distinctly in their morphologies when grown on the same medium at the same time under identical conditions. Not only are the tendencies to become yeast-like or mycelial different in degree, but the individual yeast cells and blastospores are different in shape and size. The yeast cells of cultures 582 and 805 resemble most closely those of the A.T.C.C. culture, but their mycelial tendency on galactose basal medium is less pronounced. The mycelial growth of
culture 806 is greater than is ordinarily obtained with the A.T.C.C. culture, and the blastospores and individual cells are more slender and much longer. Cultures 493 and 679 resemble the A.T.C.C. culture grown under adverse conditions. Previous morphological and physiological relationships observed on the latter strain would indicate that these organisms also have different physiological requirements.

Discussion

It is evident from the results obtained in this study, at least so far as this particular organism is concerned, that some of the factors affecting morphology given by previous authors must be somewhat modified. For the sake of clarity and convenience, these factors will be considered individually.

Influence of pH.—From the review of the literature there seems to be little agreement among the various workers concerning this factor. To the extent that extreme pH ranges exert a toxic effect which has a morphological influence on the organism, the results of this study are in agreement with those of Roux and Linossier (1890). These workers found that the toxic effect is manifested by an individualization of filaments. In the present study, however, the toxic effect of extreme pH ranges, as well as other types of toxicity, almost invariably produced yeast-like cells. As previously discussed, Talice (1930) considered this factor rather important, but that the most filamentous morphology is obtained at pH 8. According to Langeron and Guerra (1939), pH is one of the most important factors, filaments being produced in an alkaline medium, yeast cells in acid.

Since there were no precise methods employed in this study for determining relative rates or quantities of growth, the exact pH optimum is not certain. The most regular, thread-like filaments and uniformly oval yeast cells and blastospores were produced at pH 5. Increasing or decreasing the pH resulted in swollen, irregular filaments, a preponderance of yeast-like cells, and an early (2 days) appearance of the thick-walled chlamydomospores. This irregular morphology having been observed constantly in media known to be unsuitable for optimum growth, it is concluded that a slightly acid range (pH 5–6) is optimum for this organism. It is thus evident that pH is a very important factor, though the range must be varied considerably to exert a very noticeable influence. This influence is probably due to the toxicity exerted upon the organism. It is, perhaps, noteworthy also that the medium soon becomes acid when a readily assimilable carbohydrate is employed. When a carbon source not so readily assimilable is used or the source is too dilute, the medium becomes alkaline. It is considered that the real, morphology-determining factor in this case is one of nutrition, but the pH changes probably have some influence also.

Influence of Nutrients.—It is generally agreed among mycologists that this particular factor is of prime importance and that filamentation occurs as a result of starvation. The idea that "impoverished" media is necessary for the production of filaments developed as a result of growing the organism on various natural
substances of unknown chemical composition. It is apparently true that most natural media which produce the filamentous form yield a rather poor growth, whereas those which produce a yeast form usually yield a heavier growth. The results obtained on natural media in this study agree with those of previous authors. The results obtained on chemically identified media, however, do not support the general statement concerning "impoverished" media and are in direct opposition to that concerning the morphological influence of readily assimilable carbohydrates.

Of the sugars used in this study, galactose gave the heaviest mycelial growth, and maltose and sucrose were better than glucose, fructose, and mannose. With the exception of sucrose, which was never fermented (gas), there seems to be a relationship between the rate of fermentation and the amount of filamentation. Those which were most readily fermented (glucose, fructose, and mannose), though producing abundant filamentation, also produced more blastospores and yeast cells than the less readily fermented sugars. The reducing sugar content within the range of 1 to 3 per cent does not appear to have the importance in cell division that Nickerson attributed to it. Galactose, also a reducing sugar, not only produced the most abundant growth, but also the most abundant mycelium.

It has been shown that good filamentation not only can occur on fairly large concentrations of readily assimilable carbohydrates, but that they are necessary for good filamentation. In addition to the necessity of carbohydrates, potassium and biotin are also essential. An absence or deficiency of any one or all of these three substances results not only in a very poor growth, but the growth which does occur is of the soft, creamy type of yeast-like morphology. Phosphorus, though essential to the growth of the organism, does not seem to affect its filamentation to a very great extent. With more highly purified chemicals than ordinary C. P. chemicals such as those used in this work, the effect of phosphorus, as well as some of the other minor elements, would undoubtedly have been more evident. The very noticeable effect of the phosphate deficiency was the very early (24 hours) appearance of numerous chlamydospores. The fact that no other deficiency produced this effect in such a short period of time indicates that the production of chlamydospores is stimulated by the exhaustion of the available phosphorus in the medium. Another obvious feature of the organism grown on phosphate-deficient medium are the numerous, large vacuoles both in the filaments and the yeast cells.

Many mycologists have observed that natural media can be divided into two groups depending upon whether they produce a yeast-like or a filamentous growth of Candida albicans. It has been shown in this study that those substances which produce a filamentous, though a poor growth, can be fortified with carbohydrates and inorganic salts to produce good growth without affecting the morphology of the organism—that is, a heavy filamentous growth. On the other hand, no amount of fortification has been found suitable for inducing a yeast-producing natural medium to produce filaments. When a yeast-producing substance such as yeast
extract is added to a complete synthetic medium which produces abundant filamentous growth, the resulting growth is soft, creamy, and yeast-like, but little heavier than that obtained on synthetic medium alone. The results of these experiments indicate that most natural media contain various unknown substances which induce a yeast-like morphology in Candida albicans. That there is ample available carbon in these substances is shown by the rich growth which occurs upon them without additional carbon sources. It is doubtful that these substances are sugars since the metabolism of the organism brings about an alkaline reaction instead of the characteristic acid of carbohydrate metabolism. It is perhaps true that these natural media may contain so much nitrogenous material that the ammoniacal products of metabolism may mask the acidity given off by the carbohydrate metabolism. However, one pure natural substance, succinic acid, was readily utilized as a carbon source, and the medium became alkaline. The resulting morphology on this medium was yeast-like. There are doubtless other substances in natural material which serve as carbon sources for this organism and produce the yeast-like form.

It is then necessary to modify or perhaps do away with the term "impoverished" media when referring to media necessary for producing mycelium in Candida albicans, since a filamentous growth can also be a very rich growth.

Influence of temperature.—Except in liquid media where there was little detectable difference, a high temperature (37–40° C.) produced a very strong tendency toward the yeast phase. The only explanation for the discrepancy between this finding and that of other authors is that we are evidently using different organisms. If this be true, then a better description of the organism is needed, since the characteristics of this one have fulfilled all the morphological and biochemical requirements listed by the taxonomists.

Effect of the consistence of the media.—It has been observed, almost from the first study made on this organism, that the mycelial tendency is stronger in liquid than on solid media. We found this especially true in a medium which usually produced a yeast-like morphology in the solid state. The other factors, such as temperature and even nutrition, were not so obvious in their effects, though they were usually noticeable. This effect is generally attributed to the reduced oxygen tension in liquid media, but it is not so easily proven. In this study it was found that the organism could not grow anaerobically on agar. In liquid media the growth seems to occur mainly at the top and then precipitates to the bottom in a cottony mass. Indeed, if one is careful not to shake the culture tube, the mass of the organism is seen to be located in two separate places—one very fine mass at the top and the characteristic cottony mass at the bottom. The liquid between these two masses is often practically clear. By means of a dropper-type pipette, samples of each were obtained separately for microscopic examination. The examination of young cultures revealed short, highly branched chains of yeast cells at the top and long thread-like filaments at the bottom. From these experi-
ments, it seems that filaments produce clumps at the top of the medium which settle to the bottom, leaving space for individual yeast cells or blastospores to begin the process over again. The little clumps of pseudomycelium seem able to grow for a short time after sinking further into the medium, producing the typical filaments. Regardless of what the true process is, the growth cycle is not essentially different from that obtained on solid media. The filaments are produced in abundance only in young culture, and as the culture ages, the filaments degenerate until the culture becomes a granular mass composed almost entirely of yeast cells.

The effect of solid media is just as difficult to interpret as that of liquid media. Wickerham and Rettger (1939) believed that placing a cover-slip over a developing colony created the reduced oxygen tension necessary for filament formation. However, we observed the zone of filamentation consistently on the outer edge of a developing colony on petri-dish cultures which were not covered with coverslips. Observations on a giant colony reveal that the spread is accomplished by this ever-widening zone of naked filaments which soon become covered with blastospores but never covered all the way to the tips (pl. 16, fig. 12). If reduced oxygen tension favors the production of filaments and retards the production of blastospores, it is rather strange that practically all the filamentous growth is toward the outside of a colony while blastospores are produced nearer the center where competition for oxygen would be much greater. This may be observed in the samples taken at various distances from the center of a giant colony, and the effect is even more striking when a two-inch square of an agar plate is evenly streaked with a culture of *Candida albicans*. The inner zone contains practically nothing except yeast cells, while the outer zone grows like a giant colony producing a luxurious, filamentous growth upon which blastospores develop (pl. 16, fig. 10).

The above descriptions are typical of growth obtained on good filament-producing media. When a poor mycelium-producing medium is used such as succinate basal medium the results become confusing. The growth on slants, as previously observed, is almost entirely yeast-like with only occasional filaments. In a giant colony, though the center is yeast-like as expected, there is also an outer zone of filaments. However, instead of being on the surface as they are in the carbohydrate basal medium, all seem to be growing down into the agar. They become covered with a sleeve of blastospores which makes them visible macroscopically.

It is believed that this phenomenon and those previously described in liquid media have led to the conclusions in regard to anaerobism.

The relationship between the ability of the organism to produce filaments and its ability to produce gas (anaerobic fermentation) on a particular substrate should also be considered. In every case there was better mycelium production on those sugars (galactose, maltose, and sucrose) which were fermented very slowly than on those (glucose, fructose, and mannose) which were rapidly fermented. Also, in five tubes each of glucose and sucrose broth inoculated with one loop of suspension from the same inoculum and incubated in the same rack, the sucrose cul-
tures could quite easily be distinguished from the glucose because of their more abundant growth. This indicates not only that anaerobic fermentation fails to help in the production of a mycelium, but it also lowers the efficiency of the sugar utilization. From my observations it is therefore concluded that, with the proper medium and incubation at the proper temperature, comparable results are obtained on liquid and solid media.

Effects of adding various substances to the basal medium.—In general, substances not required by the yeast but which influence its morphology are of two types: (1) those which show their toxicity by retarding growth; and (2) those which do not appreciably influence the quantity of growth but influence the morphology of the organism.

* There are, of course, numerous known chemicals of the first type—phenol, various metallic ions such as cobalt, etc., if used in too high concentrations, and anions such as iodide and chloride. Their toxic effect on morphology is nearly always toward the yeast form but there are evidently exceptions. High concentrations of chlorides were found, in this study, to inhibit growth somewhat and also seemed to inhibit the development of blastospores so that a purer mycelium was obtained. This may have been the result of the high osmotic pressure exerted by these salts. High concentrations of sugar, however, have the opposite effect on morphology. Nickerson (1950) found that he could suppress the yeast cells and obtain cultures of almost pure mycelium with dilute concentrations of cobaltous nitrate. The second type of substances are chemically unidentified compounds contained in varying amounts in most natural media. The chemical separation and identification of these substances are not within the scope of this study, but their presence is easily demonstrated by adding a bit of natural material such as yeast extract to a complete basal medium and observing the change in morphology exhibited by the organism.

Morphological comparisons of various strains.—If one is to accept all of the strains of yeast-like fungi that various taxonomists have placed in the species Candida albicans, he must accept also a great variety of morphologically and probably biochemically different characteristics of the organism. Considering that there are only three criteria upon which one can base his classification—namely, production of terminal chlamydospores; fermentation of glucose, fructose, mannose, and maltose, but not sucrose; and production of filaments—there is little wonder that he is unable to choose any typical organism for his study and have the results agree with those of another mycologist supposedly working with the same organism. Mackinnon (1940) would explain most of these differences as being due to spontaneous variation or dissociation, so that if pure yeast cells are chosen as one extreme and pure filaments as the other, a given strain may have undergone any amount of dissociation which would determine its yeast to filament ratio. If one accepts this as the cause for the differences in all the so-called "strains" of Candida albicans, he must also accept the fact that the shapes of the yeast cells and blastospores change considerably. Of the Mackinnon strains, there were at least three
different cell shapes. That these strains were not all satisfied nutritionally is indicated by the swollen, knobby appearance of the filaments. Perhaps, if all of these strains were derived in his laboratory from the same culture, the organism would be so protean that it is impossible to attribute to it any more than the three characteristics given.

With the organism employed for this particular study, the results do not indicate that it is as variable as indicated by Mackinnon. It is true that when this organism was streaked on plates, there were often the two types of colonies described by Mackinnon—the prickly, firm colony that could only be removed intact and the soft, creamy colony. When these aged, however, or were broken up and transferred to slants, there was little difference in their macroscopic or microscopic appearance. Either the filamentous form or the yeast-like form of each was obtained, depending upon the medium upon which they were cultured. The requirements for filamentation were the same for each and the blastospore shape never varied.

Finally, it is observed that an organism is better characterized after the second or third transfer on a given medium in 24- to 48-hour periods. The first transfer, in many cases, does not usually produce an organism greatly different from that upon which it was previously growing, particularly if one does not wash the inoculum thoroughly before using it. It is well known that microorganisms store up some critical materials, especially certain vitamins or growth factors, in sufficient quantity to suffice them for one or two generations on media lacking these elements. The first generation, therefore, may indicate not only the effect of that particular medium, but also that of the stock medium. There is another good reason for two or three successive transfers, if one wishes to study the organism under maximum conditions. The lag phase is virtually eliminated by such frequent transfers, and the organism is maintained at its maximum growth rate.

Summary

In order to determine what factors were influential in determining the morphology of the highly variable Candida albicans, a chemically defined medium was utilized. Since this medium was readily modified in various specific ways, it was possible to attribute any morphological change to a definite change in the culture conditions. By varying not only the constituents of the medium, but also the physical factors such as temperature and consistency, quite definite conclusions could be reached. In general, it was found that Candida albicans A.T.C.C. 2091 requires for filament production a readily assimilable, but not so readily fermented carbohydrate. It also requires phosphorus, potassium, and biotin. The optimum temperature for filamentation is 25–30° C. The optimum pH is near 5. Filaments are produced most abundantly during the maximum growth phase.

The yeast-like phase results from lack or deficiency in any of the above nutrients, a high temperature (37–40° C.), especially on solid media, unfavorable pH range, and toxic substances. Many natural substances contain unidentified
products which, though not growth-inhibiting, produce the soft, creamy, yeast-like form. Yeast-like forms predominate in the lag and decline phases of a culture as the filaments undergo degeneration.

Chlamydospores are produced as a result of unfavorable conditions such as too high or too low pH, deficiency of phosphorus, and to a less extent other deficiencies which are necessary for maintenance of normal growth.

The effects of liquid media on growth, especially as it pertains to reduced oxygen tension, were indefinite. The organism grew poorly, or not at all, in an anaerobic jar on solid media. On liquid media, the growth was observed on top of the medium from whence it precipitated, leaving room for more such growth. Growth on sucrose medium which, if fermented at all, is admitted to be very slow, was considerably better than that obtained on the readily fermented glucose. The sucrose medium in every case produced the greater proportion of filaments.

**Bibliography**


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**Explanation of Plate 15**

*Candida albicans*

Fig. 1. Effect of a high pH (9). Note the numerous yeast-like cells, the chlamydospores, and the few scattered filaments, × 213. Incubated at 24° C.

Fig. 2. Growth on approximately 2 per cent succinate basal medium for 24 hours at 24° C., × 213. The same morphology is obtained on histin and carbohydrate-deficient media.

Fig. 3. Growth on a phosphate deficient basal medium 24 hours at 24° C., × 213. Note the fairly numerous chlamydospores and yeast-like cells.

Fig. 4. Rosette-like clusters of short pseudohyphae resulting from a potassium deficiency, × 213. Grown on 2 per cent sucrose basal medium at 24° C. for 48 hours.

Fig. 5. Heavy mycelial growth resulting from growth for 24 hours incubation at 24° C., × 213. Maltose medium produces the same morphology.

Fig. 6. Growth on 2 per cent glucose basal medium for 24 hours at 24° C., × 213. Mannose and fructose media produce the same morphology.