LIPID PRODUCTION BY A SOIL YEAST

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It has been well established that fatty substances are produced by various microorganisms, notably by certain yeasts and filamentous fungi as well as by the tubercle bacilli and a species of *Azotobacter*. The tubercle bacilli and other acid-fast bacteria contain from 20 to 40 per cent lipid on the dry-weight basis (Anderson, 1939). Even some of the common non-acid-fast bacteria may contain considerable amounts of lipid. *Escherichia coli*, *Staphylococcus albus*, and *Bacillus megatherium* were found to contain from 8 to 40 per cent lipid when grown on certain media (Larson and Larson, 1922). More commonly, however, the lipid content of bacteria has been reported to be below 10 per cent. The results of Gorbach and Sablatnög (1934*a*, 1934*b*) may be cited in this connection. They found that whereas the lipid content of *Pseudomonas aeruginosa* was 0.6 per cent when grown on meat extract agar at pH 7.0, there was as much as 3.9 per cent lipid when the organism was cultivated on mannitol. Anderson (1939) reported 2 to 6 per cent lipid in cells of *Phytomonas tumefaciens* and 7 per cent in *Lactobacillus acidophilus*.

Among the bacteria, *Azotobacter indicum* is unique in lipid production (Starkey and De, 1939). The cells of this bacterium commonly contain two large fat globules, one at each end of the rod-shaped cells, and as much as 50 per cent of the cell volume is occupied by the fat globules.

The lipid content of filamentous fungi ranges between 1 and 40 per cent, and differs with the various cultures and the conditions under which they are grown. (Pruess, Eichinger, and Peterson, 1934; Prescott and Dunn, 1940; Bloor, 1943). The lipid content of 24 filamentous fungi studied by Pruess and Strong (1933) was from 1 to 25 per cent with an average between 6 and 9 per cent. Ward, Lockwood, May, and Herrick (1935) determined the lipid content of 61 fungi and reported that six of the fungi contained over 20 per cent lipid. Under favorable conditions of cultivation, *Penicillium javanicum* was found to contain as much as 41.5 per cent. Large amounts of lipid were produced by species of *Oospora* Wallroth when grown on milk, and the results of Geffers (1937) indicate that this organism can be used to synthesize fat from milk wastes. Up to 50 per cent of the mycelium could be extracted with fat solvents.

Attention was focused on lipid production by yeasts and related organisms through the results of Lindner and associates, who developed a process for the production of fat from carbohydrates by *Endomyces vernalis* in Germany during World War I (Fink, Haehn, and Hoerburger, 1937; Prescott and Dunn, 1940). When cultivated under conditions favorable for lipid production, this yeast con-

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tained as much as 44.7 per cent lipid; but when cultivated on a production scale, there was about 20 per cent lipid. Heide (1939), who recently studied the influence of medium composition on lipid production by *Endomyces vernalis*, found as much as 42 per cent lipid in the cells under conditions favorable for lipid accumulation.

Several yeasts were studied by Rippel (1943) for lipid production, among which was *Nectaromyces reukaufii*, which produced 10 to 15 g of lipid for each 100 g of carbohydrate decomposed.

The results of Heide (1939) and others (Bichkovskaya, 1939; Prescott and Dunn, 1940; Raaf, 1941–1942; Rippel, 1943) indicate that conditions favorable for maximum lipid content of yeast are not those giving the maximum conversion of the carbon source of the substrate to lipid. Cells grown in media rich in nitrogen give high yields, but their lipid content is low; media deficient in nitrogen give low yields of cell material, but the cells contain an abundance of lipid. As stated by Smedley-MacLean (1922), there is generally an inverse relationship between the quantity of yeast cells produced on various media and the percentage of lipid in the cells.

The relative concentrations of carbohydrate and nitrogenous materials in the media must therefore be carefully balanced to obtain the maximum yield of lipid. Among the other factors affecting lipid production are the reaction of the medium, phosphate concentration, degree of aeration, nature of the carbon source, and the presence of certain metallic ions. Not only are there great differences in the potentialities of different microorganisms for lipid production but the composition of the medium and the environmental conditions likewise have a profound effect on lipid production by any one culture. The substrates which have been proposed for lipid production by yeasts and filamentous fungi include milk waste, molasses, waste sulfite liquor, cellulose waste, and hydrolyzed wood.

Methods have also been developed for the production of yeast as a source of protein. The yeast is cultivated in media containing carbohydrate and supplied with nitrogen from ammonium salts. Thaysen (1943) and Thaysen and Morris (1943) recently reported results of studies with *Torulopsis utilis* from which it was concluded that this "food yeast" which was rich in protein and vitamins could be produced very economically (Gortner and Gunderson, 1944). New strains were developed which grew well at relatively high temperatures and produced cells larger than the original strains, thus facilitating production and processing.

EXPERIMENTAL

The yeast with which this report is concerned was encountered during studies of the nitrogen-fixing population of soils and has been recovered from soils periodically since 1935.[2] The yeast cultures were obtained from colonies which developed on a "nitrogen-free" agar medium having the following composition: glucose, 15 g; K_2HPO_4, 0.8 g; KH_2PO_4, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.2 g;

[2] A preliminary report was presented before the Theobald Smith Society (Starkey, 1944).

$CaCl_2 \cdot 2H_2O$, 0.05 g; $Na_2MoO_4 \cdot 2H_2O$, 0.0005 g; $MnSO_4 \cdot 2H_2O$, 0.0005 g; $Na_2WO_4 \cdot 2H_2O$, 0.0005 g; $FeSO_4 \cdot 7H_2O$, 0.025 g; agar, 15.0 g; and distilled water, 1,000 ml. A small amount of the soil being tested was scattered over the surface of plates of the solidified agar medium and incubated at 28 C. The common types of *Azotobacter*, if present, usually developed within 2 to 3 days and were characterized by their glistening, soft, smooth, raised colonies. When the incubation period was prolonged to from 7 to 10 days, other colonies appeared which resembled those of *Azotobacter*. They were raised and glistening and had the appearance of very thin starch paste. They were watery, tended to spread, and sometimes coalesced, forming a thin watery film over a considerable portion of the plate. When examined under the microscope, this material was found to contain large, nearly spherical cells approximately 8 μ in diameter suspended in thin slime. Some of the cells had a single smaller spherical bud, but there was no tendency toward the formation of large aggregates. Each of the cells was found to contain a large, highly refractive globule which nearly filled the cell (figures 1, 2, and 3).

The watery, spreading colonies were consistently obtained from various soils including those from cultivated and uncultivated fields, from pastures, waste land, and woodland. The soils varied in reaction from pH 4.0 to 7.5. The cells which composed the colonies all had the same appearance and were characterized by the presence of large refractive globules, one in each cell. The regularity with which the yeast was encountered suggests that the organism is a common soil inhabitant.

When isolated in pure culture and cultivated on slants of the glucose, nitrogen-free, agar medium, the yeast cultures continued to produce large spherical cells nearly filled with the large refractive globules. The cell material had a watery consistency and tended to settle to the base of the slant.

The appearance of the globule suggested that it was lipid, and tests indicated that this was the case. When the cell material was treated with Sudan III, the globules stained red, whereas the remainder of the cell was colorless. The lipid globules of cells which were treated with warm dilute acid to hydrolyze the slime stained more readily. The fact that large amounts of fatty material were obtained by extracting the cells with fat solvents, as well as the fact that the cells no longer became colored with Sudan III after the extraction, proved that the globules were lipid.

Sporulation. The yeast is of interest not only by reason of its high lipid content but because of its unusual spore formation. The following sequence of changes was observed with cultures developing in association with other microorganisms on the glucose, nitrogen-free, agar medium which had been inoculated with soil. During the first two weeks the cells consisted entirely of the spherical cells with spherical buds, nearly filled with globules of lipid. During the next few days some of the yeast cells produced buds of a different type. They were shaped like a sac and contained material which had a granular appearance in contrast to the homogeneous, refractive lipid globules in the spherical buds (figures 4 and 5). In a few more days the granular substance became trans-

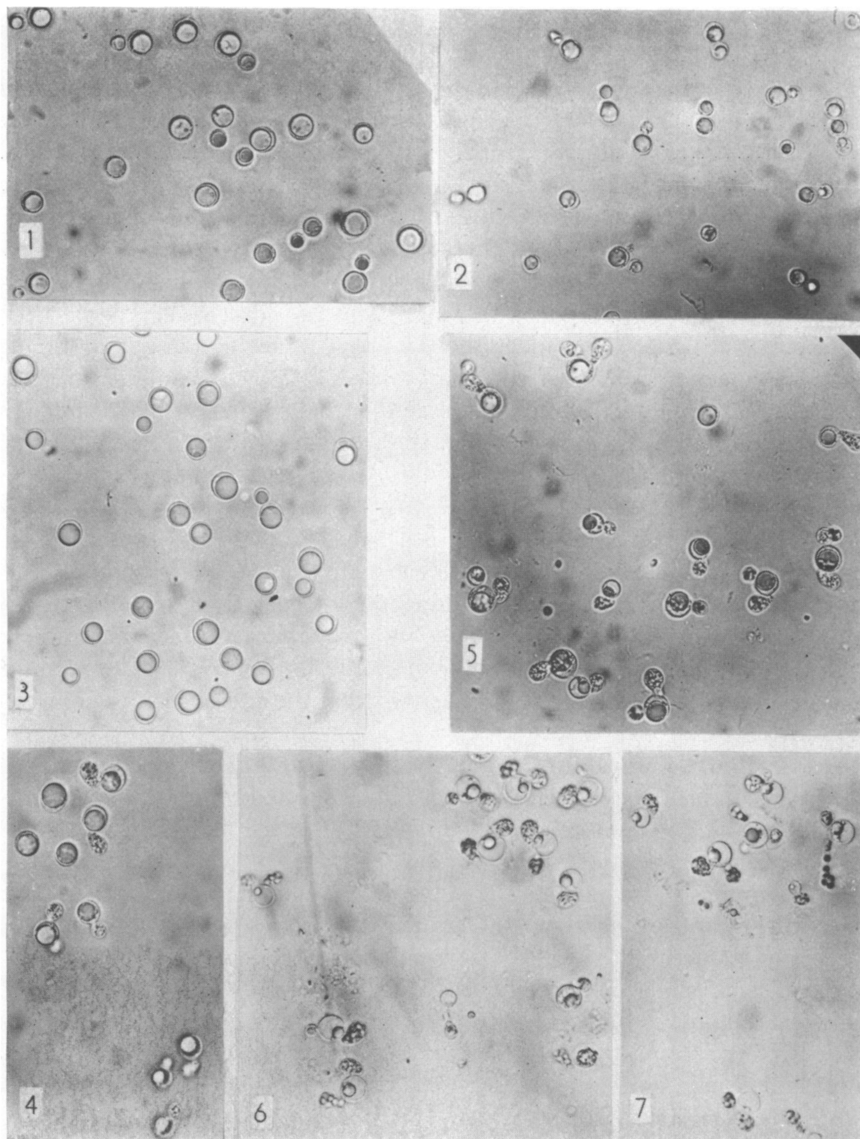

FIG. 1. CELLS FROM A COLONY ON A NITROGEN-FREE, GLUCOSE, AGAR PLATE SEEDED WITH SOIL

Each spherical cell is nearly filled with a single large fat globule. One cell at lower right has a hazy outline caused by the breaking away of an outer cell membrane. Culture age 13 days. Magnification × 330.

FIG. 2. CELLS OF A PURE CULTURE OF STRAIN 72 GROWN ON GLUCOSE, NITROGEN-FREE AGAR

There is a fair-sized single fat globule in each cell. Culture age 6 days. Magnification × 330.

FIG. 3. CELLS OF A PURE CULTURE OF STRAIN 72

Typical spherical cells each nearly filled with a single fat globule. Culture grown on nitrogen-deficient aerated solution medium. The cells were taken from one of the aerated solutions used for tests of fat production by the yeast. Culture age 20 days. Magnification × 330.

formed into ascospores (figures 6, 7, 8, and 9). The ascus remained attached to the parent cell even after spore formation was completed, and additional vegetative buds were frequently seen attached to the parent cell. In some cases the parent cell bore more than one ascus. In no case was there evidence of spores within the parent cell. The globule of lipid persisted in the parent cell throughout the period of spore formation, but it became reduced in size as the incubation period was prolonged.

Many yeasts, two of which were characterized by the presence of large fat globules, were isolated from soil by Starkey and Henrici (1927). These were described as *Nadsonia fulvescens* and *Torulaspora* sp. The descriptions of these do not characterize the yeast under consideration.

Spore formation has not been observed with pure cultures grown on the glucose, nitrogen-free, agar or similar media containing 0.01 per cent yeast extract. The medium containing 0.01 per cent yeast extract was similar to the medium from which the yeast cultures were isolated, but differed in that it contained no sodium chloride, yeast extract, or salts of manganese, molybdenum, and tungsten. The modified medium with 0.01 per cent yeast extract is the one subsequently designated as the nitrogen-deficient medium. Spores were produced, however, on several media. On the vegetable infusion agar of Mrak, Phaff, and Douglas (1942) spores were first noted with cultures 14 days old, and the asci became more numerous during the incubation period of 2 months. No spores were produced by cultures grown in a solution medium of similar composition. Spores were produced by cultures which were heavily inoculated on media unfavorable for growth, such as nitrogen-deficient agar media in which rhamnose, lactose, ethanol, or glycerol was substituted for glucose. On these media there was no apparent growth in some cases and poor growth in others, but spores were produced. Spores were observed with some cultures after 7 days' incubation on media containing rhamnose, lactose, or glycerol, and there was more sporulation after 27 days.

Nickerson and Thimann (1941) found that material produced by *Aspergillus niger* on malt extract medium enhanced conjugation and spore formation by

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FIG. 4. CELLS FROM A COLONY ON A NITROGEN-FREE, GLUCOSE, AGAR PLATE SEEDED WITH SOIL

Several of the cells show saclike buds filled with granular material and attached to the parent cells, each of which is nearly filled with a single large fat globule. Some fat-filled cells free from buds. Culture age 17 days. Magnification × 330.

FIG. 5. CELLS FROM THE SAME CULTURE AS FIGURE 4

Many cells show saclike buds filled with granular material and attached to the parent cells, which have large fat globules. Some cells have 2 buds each. Culture age 17 days. Magnification × 330.

FIG. 6. CELLS FROM THE SAME COLONY ON NITROGEN-FREE, GLUCOSE, AGAR PLATE SEEDED WITH SOIL AS THAT FROM WHICH CELLS OF FIGURES 4 AND 5 WERE OBTAINED

The cells show a transition stage in spore formation. Some of the saclike buds are filled with granular material, whereas other buds contain spores, up to 8 in number. Culture age 20 days. Magnification × 330.

FIG. 7. SAME AS FIGURE 6

One cell at upper right shows an elongated ascus with eight easily distinguished spores. Asci attached to other cells show various stages in spore formation. Culture age 20 days. Magnification × 330.

certain species of *Zygosaccharomyces*. Similar material was added to glucose, nitrogen-deficient, solution media which were inoculated with the lipid-producing yeast to test for spore formation. Other solution media received additions of malt extract, yeast extract, and grass extract. In none of these solutions was there evidence of spore formation even after incubation for several months. In some cases solution media supporting growth of the yeast were inoculated with cultures of *Aspergillus* and *Penicillium*, but the associative development of the fungi failed to result in spore formation by the yeast.

Some strains produced 4 spores in each ascus, whereas in other strains there were as many as 8 spores (figures 6 and 7). One strain produced still larger numbers of spores (strain 74). The vegetative cells of this strain were larger than those of the other strains; 16 or more spores have been seen in the asci, and it is probable that the number was greater than this, since it was difficult to determine the exact number of spores which were enclosed in dense aggregates in the asci (figures 10, 11, 12, and 13). The number of spores in each ascus varied as the composition of the medium was changed, but strain 74 generally had larger numbers of spores than the other strains which were studied.

The spores had a light amber color, and the asci showed some contrast in color with the vegetative cells. The spores were oval in shape and smooth on the surface, and had a size of 2.5 to 3.3 x 3.6 to 4.6 μ, with an average of 3.0 x 4.3 μ.

Size and shape of vegetative cells. The vegetative cells varied greatly in size, but when the cultures were grown on a nitrogen-deficient, carbohydrate medium the fully developed cells of most strains had diameters of 8.0 to 9.5 μ. One strain (no. 74) consistently produced larger cells, which had diameters of 9.8 to 10.5 μ.

Under certain cultural conditions the cultures produced cells which were variable in shape. When cultivated on malt extract agar or on 2 per cent glucose agar containing 0.1 per cent ammonium sulfate or asparagine, the cells were low in fat content. During the first few days of incubation the cells were mostly spherical, but later they showed variation. They were elongated and swollen and varied greatly in size. On all media the cells multiplied by multipolar budding. Cells of cultures grown on nitrogen-deficient, carbohydrate media generally had no more than one bud. The buds freely separated from the cells grown in these media, but on malt extract agar and on agar media containing larger amounts of nitrogen the cells frequently occurred in clusters.

The cells frequently shed their outside membranes. This coating split and was shed in one piece like the coating on a bean seed, with no apparent deterioration of the cell (figure 14).

Mycelium production. The cultures produced no typical mycelium and showed no tendency toward mycelium production on most carbohydrate media. Some rudimentary pseudomycelium was formed, however, in old cultures on agar media containing rhamnose, glycerol, or mannitol. There was good growth on mannitol but very little development on the other two media. The mycelium consisted of short, distorted, hyphal projections from the round cells, and some of these short hyphae were branched.

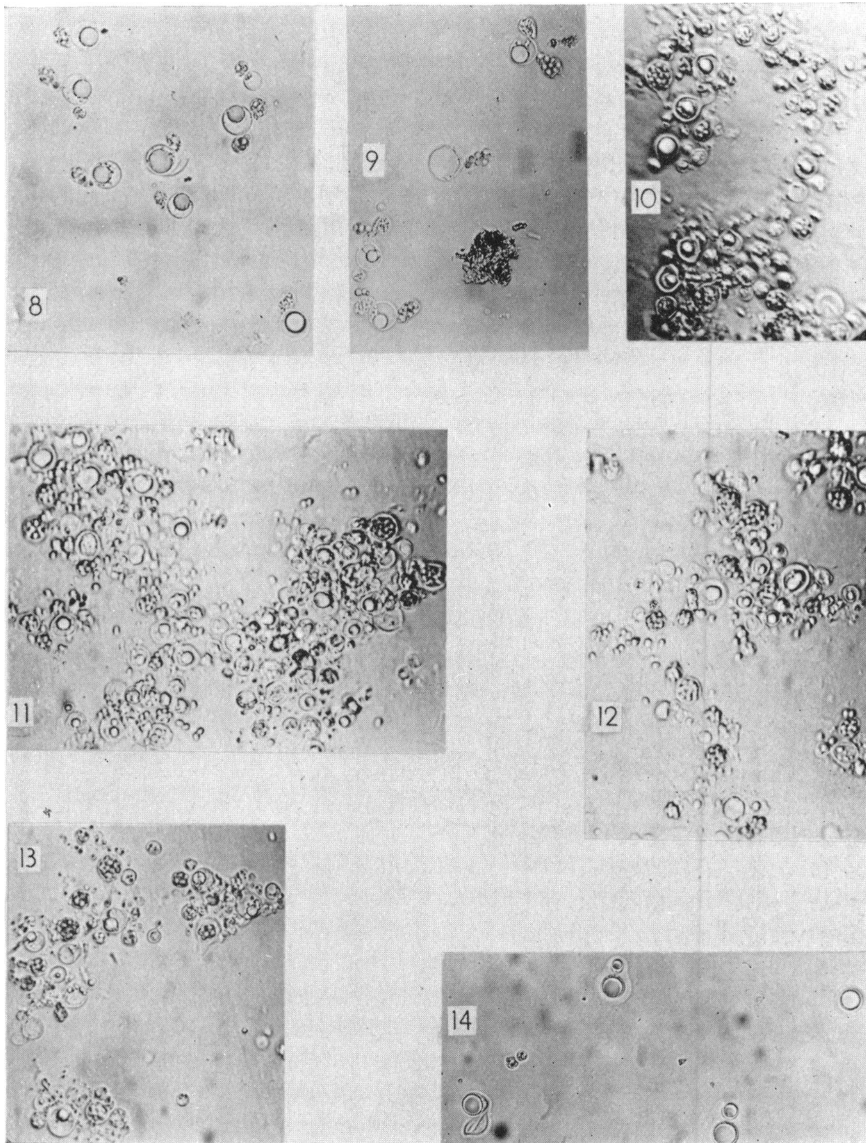

Fig. 8. Same as Figure 6

One cell in the center shows the initial stage of loss of the outer cell membrane

Fig. 9. Same as Figure 6

Fig. 10. Old Cells of Strain 74 Grown on a Nitrogen-deficient, Agar Medium Containing 0.5 Per Cent Ethanol

Most of the vegetative cells had degenerated. Many cells show spores. One cell at upper left shows many spores, 14 of which can be counted. Some cells still show presence of fat. Culture age 28 days. Magnification 330.

Fig. 11. Same as Figure 10

Some of the cells contain many spores

Fig. 12. Same as Figure 10

Fig. 13. Same as Figure 10

Fig. 14. Cells from a Nitrogen-free, Glucose, Agar Plate

One cell at lower left shows a cap-shaped outer membrane breaking away from the cell. Culture age 13 days. Magnification × 330.

Type of growth on agar and solution media. On glucose, nitrogen-free agar the organism grew slowly and produced glistening, white, watery slime resembling thin starch paste. On a similar medium containing 0.01 per cent yeast extract the cultures grew more rapidly and produced more cell material. On 2.5 per cent malt extract agar there was abundant brownish-gray, raised, spreading, slimy growth. On the plant infusion agar of Mrak *et al.* (1942) the cultures grew very rapidly and produced dark brown, moist, raised, spreading growth. In all cases the cells were imbedded in slime which caused the cell material to settle to the base of the slants. On agar slants in which various carbohydrates were substituted for glucose, the growth characteristics were the same; the cultures produced white, glistening, watery growth having the appearance of starch paste. On glycerol agar the growth was not mucoid but relatively free from slime; the cell material was first white but later turned slightly brown in color. On 2 per cent glucose agar containing 0.1 per cent asparagine there was abundant white, mucoid growth. There was even more growth when 0.1 per cent $(NH_4)_2SO_4$ was used as the source of nitrogen, but the cultures produced very little slime on this medium.

The yeast cultures made very poor growth on static solution media; no pellicle was formed and the solutions showed only slight turbidity. Most of the cell material accumulated as sediment. Since development of the organism is characterized by oxidative and not fermentative changes, the submerged cells grew slowly. Growth was very much increased by aeration, and in aerated culture solutions the yeast cells were uniformly dispersed and produced a white to cream-colored turbidity. These results are in agreement with those of Smedley-MacLean and Hoffert (1923, 1926), who reported that lipid storage is favored by vigorous aeration. Their experiments are not strictly comparable, however, since they studied the production of lipid by yeast cells suspended in solutions containing various carbon sources but no nitrogen, and under these conditions growth was suppressed almost completely.

Utilization of carbohydrates and some related compounds. The ability of the yeast to use various substances as energy sources was tested on both agar and solution media having a composition similar to that of the nitrogen-deficient medium but with various carbon compounds substituted for the glucose. The cultures were examined at various periods during the 26 days of incubation. Growth was meagre on all the solution media, but it was more abundant on the agar slants. The same substances supported growth on both agar and solution media. The yeasts grew well on agar slants containing glucose, fructose, galactose, sucrose, mannose, maltose, dextrin, starch, inulin, mannitol, and ethanol. The yeast grew poorly on glycerol. Two of the four strains tested made a small amount of growth on rhamnose; the other two failed to grow. None of the strains grew on lactose.

Glucose was not fermented. Presumably, therefore, none of the carbohydrates is fermented (Henrici, 1941).

Utilization of nitrogen compounds. Although the cultures were isolated from media to which no nitrogen had been added (glucose, nitrogen-free agar), the yeast was unable to fix atmospheric nitrogen. It was cultivated on nitrogen-free

solution media containing 1.5 per cent glucose or mannitol as sources of carbon and on similar media containing 0.01 per cent yeast extract. Some of the cultures were aerated and others were static. In none of the media was there any nitrogen fixation by the yeast, although there was fixation of 9 to 20 mg of nitrogen by cultures of *Azotobacter* grown in 100-ml portions of similar media.

The yeast utilized ammonium salts, asparagine, and yeast extract as sources of nitrogen. Nitrate was not utilized.

Gelatin was not liquefied in gelatin stabs containing 2 per cent glucose, and there was no decomposition of gelatin in gelatin-agar plates containing 2 per cent glucose (Frazier, 1926) during 18 days of incubation.

Influence of medium composition on lipid production. The lipid content of the cells was greatest when the cultures were grown on nitrogen-deficient carbohydrate media. The lipid globules were small in cells grown on malt extract agar, the vegetable infusion agar of Mrak *et al.* (1942), and agar media containing 2 per cent glucose and 0.1 per cent asparagine or $(NH_4)_2SO_4$. As has been repeatedly noted by others with cultures of yeast and filamentous fungi, the nitrogen content of the medium had a pronounced effect on lipid production. In media with relatively large amounts of available nitrogen per unit of carbohydrate, lipid accumulation was suppressed. Nitrate had no effect on lipid accumulation. This apparent anomaly is explained by the fact that the yeast did not utilize nitrate. It has been observed that the cells frequently contained smaller fat globules during the first few days of incubation than at later stages of growth.

Cells in static solution media contained relatively small amounts of lipid, whereas they were nearly filled with lipid when cultivated in aerated media of the same composition. The cultures grew well and produced large amounts of lipid in aerated solution media containing 3 per cent glucose, 0.05 per cent $(NH_4)_2SO_4$, and 0.01 per cent yeast extract.

All of the yeast strains which have been isolated appeared to be the same except for minor differences. The yeast is characterized by slow growth in solution media, lack of fermentation, distinctive means of sporulation, and high lipid production under suitable cultural conditions.

Classification. The morphological and physiological characteristics of the yeast are such that the organism is not readily classified. Wickerham concluded that, except for spore formation, it is in many respects similar to some members of the asporogenous yeasts of the genus *Torulopsis* (Wickerham, 1944).

A culture isolated by den Dooren de Jong (1926) and named *Torula lipofera* resembles the yeast morphologically and physiologically, except for spore formation. The name of the organism was subsequently changed to *Torulopsis lipofera* by Lodder (1934). The yeast was isolated from soil, produced cells with large fat globules when grown on suitable media, did not ferment carbohydrates, produced no pellicle, and grew on similar carbohydrates, alcohols, and nitrogenous materials (Lodder, 1934). Since there is such a close similarity between *T. lipofera* and the yeast isolated from New Jersey soils, cultures of *T. lipofera* might profitably be re-examined for spore formation.

The yeast might be classified under the family Endomycetaceae, subfamily

Saccharomycoideae, and tribe Saccharomyceteae, but it cannot be classified as to the genus with certainty.[3] In fact, it shows many characteristics which suggest closer agreement with some of the asporogenous yeasts than with the well-characterized genera of sporeforming yeasts.

On the basis of preliminary observations, Wickerham (1944) concluded that it was unlikely that the yeast belonged in any of the present genera of sporogenous yeasts since none of the latter contain yeasts with its characteristics, such as mucoid cell material, colored ascospores, ascospores in excess of eight when cultured on certain media, and a strong tendency of old cells to shed their outer membranes. In some respects the yeast resembles a culture described as *Debaryomyces hominis* by Todd and Herrmann (1936). However, the structures which were described as spores of *D. hominis* are different from the spores of the soil yeast, but comparative studies might show more similarity between the two than is apparent from the description of Todd and Herrmann.

Additional information is needed before the yeast can be completely classified and before it is possible to determine with certainty whether it is identical with any yeast which has already been described or is a new species.

Efficiency of conversion of carbohydrate to lipid. The yeast was cultivated in volumes of medium varying from 500 to 7,000 ml, in order to determine the amount of lipid contained in the cells and the relative portion of the carbohydrate which was converted to lipid. The medium commonly used had the following composition: tap water, 1,000 ml; glucose, 15 g; yeast extract, 0.1 g; K_2HPO_4, 0.8 g; KH_2PO_4, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.05 g; and $FeSO_4 \cdot 7H_2O$, 0.025 g. The carbohydrate was sterilized separately from the rest of the medium. Since preliminary tests indicated that growth was much greater in aerated media, a vigorous stream of sterile air was bubbled through the medium throughout the period of incubation. The same yeast culture (strain 72) was used in all these experiments.

Extraction of lipid from the cells. The yeast was cultivated in 500 ml of medium, and the cell material was harvested after 5 days' incubation. It had grown well and produced a heavy, cream-colored suspension of cells which contained large lipid globules. The cells were removed by centrifugation, and the white, creamy cell paste was dried in a current of air at 50 C. After most of the water was removed, the material was dried at 60 to 70 C. Determinations were made on the medium for residual carbohydrate and reaction, and a portion of the cell material was tested for ash content. The rest of the cell material was extracted with ethyl ether. Only a small amount of material was obtained in the extract. The dried cell material was then extracted with chloroform, which removed less material than the ether. Chloroform extraction was followed by extraction with a boiling mixture of equal parts of ethyl ether and ethanol. A

[3] Dr. L. J. Wickerham of the Northern Regional Research Laboratory at Peoria, Illinois, and the late Dr. A. T. Henrici offered many helpful suggestions and aided materially in characterizing the yeast and clarifying its taxonomic status. They have verified many of the results which are herein reported and have testified to the difficulty of identifying the yeast with any of the known species.

small additional amount of extract was obtained. The total amount of material extracted by the three solvents was 11.0 per cent of the initial cell weight. After each extraction the cell material was treated with the fat stain, Sudan III, and examined microscopically. None of the solvents altered the appearance of the lipid globules, which stained as well with Sudan III after extraction as before being treated. In an attempt to release the lipid, the cell material was hydrolyzed with 2 per cent HCl at 100 C for 4 hours. The acid was then neutralized, and the mixture was extracted with ether. An amount of material equal to 42.3 per cent of the original substance was extracted. The extract was a light-amber-colored fatty substance having a low melting point. As shown by the data in table 1, 53.3 per cent of the original cell material was extracted by the

TABLE 1

*Recovery of lipid from yeast cells**

Reaction of medium	
Initial pH	7.0
Final pH	3.8
Glucose content	
Initial	6.350 g
Final	0.110 g
Glucose utilized	6.240 g
Yeast cells	1.828 g
Conversion of glucose to yeast cells	29.3%
Ash content of cells	3.1%
Extraction of lipid from cells:	
1. Initial ether extract	3.2%
2. Extracted with chloroform after treatment 1	0.6%
3. Extracted with boiling mixture of 50–50 ether and ethanol after treatment 2	7.2%
4. Extracted with ether after hydrolysis with hot 2% HCl following treatment 3	42.3%
Total material extracted	53.3%
Conversion of glucose to lipid	15.6%

* Incubation period 5 days; aerated medium containing 0.01% yeast extract and 1.5% glucose.

solvents. Most of the glucose had been consumed, and 15.6 per cent as much lipid was recovered as glucose used. The yield of cell material was 29.3 per cent of the weight of the glucose used. The reaction of the medium became distinctly acid and dropped from an initial pH of 7.0 to 3.8.

Smedley-MacLean (1922) also found that only a small portion of the lipid of yeast cells with which she worked could be obtained by direct ether extraction. Several times as much lipid was obtained after hydrolysis with HCl (boiling normal HCl for 2 hours). She also reported that similar results had been obtained by Naegeli and Loew in 1878. Most of the yeast lipid was believed to be bound up in the cells with sterol, protein, and carbohydrate and became liberated on acid hydrolysis; a smaller part of the lipid which existed as free fat could be extracted directly with ether.

Yeast growth and lipid production. One 5-liter and two 2-liter portions of medium similar to the above but containing 3 per cent glucose were inoculated with the yeast and aerated for 33 days before harvesting. At the time of harvest the cultures were composed of typical large spherical cells each containing a single large lipid globule. It was unexpected to find the cells so well preserved after such a long incubation period. The cells were separated from the 5-liter portion of medium by centrifugation. The other two cultures were extracted without

TABLE 2

*Lipid production in glucose, nitrogen-deficient medium**

	CULTURE A	CULTURE B	CULTURE C
Period of incubation, days	33	33	33
Volume of medium, ml	5,000	2,000	2,000
Reaction, pH			
Initial	7.0	7.0	7.0
Final	3.0	2.9	2.9
Glucose content			
Initial	127.0 g	50.8 g	50.8 g
Final	22.2 g	0.8 g	5.0 g
Glucose utilized	104.8 g	50.0 g	45.8 g
Yeast cells	21.175 g		
Conversion of glucose to yeast cells	20.2%		
Lipid extracted			
From cells	12.658 g		
From medium	0.227 g		
Total	12.885 g	5.811 g	5.144 g
Conversion of glucose to lipid	12.3%	11.6%	11.2%
Lipid content of cells	60.9%	57.5%†	55.6%†

* Aerated medium contained 3% glucose and 0.01% yeast extract.

† Calculated on basis of conversion of glucose to cells with culture A.

first separating the cells from the medium. The procedures were similar to those used in the preceding experiment except that the cell material and media were hydrolyzed with 2 per cent HCl and then neutralized before extraction, and ether was the only solvent used. Since the cells were not all recovered from culture A by centrifuging, both the medium as well as the separated cells were hydrolyzed and extracted. The results are reported in table 2. Most of the carbohydrate had been consumed by all three cultures. The conversion of glucose to cell material by culture A was 20.2 per cent, which was somewhat lower than the conversion in the experiment reported above. The lipid content of the cells, 60.9

per cent, was very high. Since the cells were not harvested from cultures B and C, the actual lipid content of the cells could not be calculated. An approximation can be obtained by assuming the same conversion of glucose to cells as in culture A (20.2 per cent). On this assumption, the lipid contents of cultures B and C are 57.5 and 55.6 per cent, respectively—values close to that for culture A. The conversion of glucose to lipid was similar with all three cultures and varied from 11.2 to 12.3 per cent. The reaction of the media became distinctly acid

TABLE 3

*Lipid production by yeast**

	CULTURE A	CULTURE B	CULTURE C	CULTURE D
Period of incubation, days	12	19	25	32
Volume of medium, ml	5,000	7,000	5,000	7,000
Reaction, pH				
Initial	7.0	7.0	7.0	7.0
Final	4.0	3.2	3.0	3.1
Glucose content				
Initial	127.0 g	177.8 g	127.0 g	177.8 g
Final	63.3 g	64.9 g	20.7 g	44.7 g
Glucose utilized	63.7 g	112.9 g	106.3 g	133.1 g
Yeast cells	14.93 g	25.81 g	20.60 g	22.31 g
Conversion of glucose to yeast cells	23.4%	22.9%	19.4%	16.8%
Lipid extracted				
From cells	8.54 g	14.33 g	12.45 g	13.94 g
From medium			2.07 g	3.15 g
Total	8.54 g	14.33 g	14.52 g	17.09 g
Conversion of glucose to lipid	13.4%	12.7%	13.7%	12.8%
Lipid content of cells	57.3%	55.5%	60.4%	62.5%

* Aerated medium containing 3% glucose and 0.02% yeast extract.

during yeast growth, changing from an initial pH of 7.0 to pH 2.9 to 3.0. It is of interest that the conversions were so high in view of the long period of incubation.

The results of an additional experiment are presented in table 3. The cultures were grown in a medium similar to that used previously but containing 3 per cent glucose and 0.02 per cent yeast extract. Each of two containers held 5 liters of medium and two others each held 7 liters. One culture was harvested at each of the following incubation periods: 12, 19, 25, and 32 days. Throughout the periods of incubation the media were aerated.

The yeast cells of all cultures were filled with lipid at the time of harvest. The

cultures harvested at the 25- and 32-day periods showed some signs of deterioration, however. Many cells were shedding their outer membranes, and there was considerable turbidity of the medium after centrifuging. This turbidity was probably due to disintegration of some of the cells. There was some turbidity of the centrifuged liquid from cultures A and B but more from C and still more from D. In order to recover the lipid as completely as possible, both the cell material and the centrifuged liquid of cultures C and D were extracted with ether after being hydrolyzed with HCl and then neutralized. The extract obtained from the centrifuged liquid was 14 per cent of the total obtained from culture C and 18 per cent of the total from culture D.

The results are similar to those of the other experiments: (1) the reaction became strongly acid during growth; (2) there was between 16.8 and 23.4 per cent as much cell material as glucose consumed, and the efficiency of conversion was greatest at the shortest incubation period and decreased as the period was increased; (3) the amount of lipid extracted was equal to from 55.5 to 62.5 per cent of the total weight of the cells; and (4) there was from 12.7 to 13.7 per cent as much lipid produced as glucose consumed.

The results as a whole indicate that the yeast is a relatively efficient converter of carbohydrate to lipid and that the yields compare favorably with those reported for other yeasts and filamentous fungi (Fink, Haehn, and Hoerburger, 1937; Geffers, 1937; Heide, 1939; Pruess and Strong, 1933; Raaf, 1941–1942; Rippel, 1943; Ward, Lockwood, May, and Herrick, 1935). During the course of the studies some of the cultures made relatively slow growth. The factors affecting the rate of growth and the efficiency of the conversion of carbohydrate to lipid have not been examined completely, but it is probable that the rate of growth can be accelerated and the efficiency of conversion increased by modifying the medium and changing the cultural conditions. Among the factors which may be expected to influence the transformation are the size of the inoculum, the nature and concentration of the source of carbon and nitrogen, the rate of aeration, and neutralization of the acid produced during growth.

The influence of the nitrogen content of the medium was shown by an experiment in which one medium contained 0.01 per cent yeast extract as the only source of nitrogen and a second medium contained 0.05 per cent $(NH_4)_2SO_4$ in addition. The cultures were grown in 5-liter portions of medium which were aerated during incubation for 20 days at 28 C. The results reported in table 4 are similar to those obtained in the previously reported experiments.

There was marked increase in acidity, and the final reaction of the medium containing ammonium sulfate was pH 2.6. The yeast extract medium contained inadequate nitrogen, as indicated by the fact that only 54 per cent of the carbohydrate was utilized; 89 per cent of the carbohydrate disappeared from the medium containing ammonium sulfate. Of particular interest is the efficiency of conversion of carbohydrate to cell material and lipid. The conversion to cell substance was much more efficient in the ammonium sulfate medium but the lipid content of the cells was much higher in the nitrogen-deficient medium, in which case the cells had the remarkably high lipid content of 65 per cent. The

cells grown in the ammonium sulfate medium contained 48 per cent lipid. The actual percentage of conversion of glucose to lipid was similar in both media, 10.3 per cent in the nitrogen-deficient medium and 11.6 per cent in the medium which contained ammonium sulfate, but there was much more total lipid in the latter medium.

The fact that under suitable cultural conditions there are rapid growth, nearly complete consumption of carbohydrate, and high-percentage conversion of carbohydrate to lipid suggests that the yeast might be cultivated for the production of fat from carbohydrate.

Kleinzeller (1944) made detailed studies of the conversion of carbohydrate to lipid by a similar and possibly identical yeast, *Torulopsis lipofera*. He reported

TABLE 4

*Influence of nitrogen content of medium on conversion of glucose to lipid**

	NITROGEN SOURCE	
	Yeast extract	Yeast extract and $(NH_4)_2SO_4$
Period of incubation, days	20	20
Volume of medium	5,000	5,000
Reaction, pH		
Initial	7.0	7.0
Final	3.7	2.6
Glucose content		
Initial	148.5 g	148.5 g
Final	68.7 g	16.7 g
Glucose utilized	79.8 g	131.8 g
Yeast cells	12.55 g	31.58 g
Conversion of glucose to yeast cells	15.7%	24.0%
Lipid extracted from cells	8.19 g	15.24 g
Conversion of glucose to lipid	10.3%	11.6%
Lipid content of cells	65.3%	48.3%

* Aerated medium containing 3% glucose and 0.01% yeast extract.

that under favorable conditions the yeast converted 42 to 48 per cent of the carbohydrate to yeast cells (dry weight) which contained 18.6 to 43 per cent lipid. Fat formation was highest at pH 5.5 to 6.0 and at 20 to 25 C. The high nitrogen content of the medium depressed fat formation.

Nature of the yeast lipid. No tests have been made of the chemical composition of the lipid. The mixture as a whole had a low melting point. It persisted as a soft solid at 20 C and melted slowly as the temperature was raised until most was molten at 28 C; there were still some suspended solid particles at 35 C. Upon cooling there was no solidification at 18 C, but most of the lipid was solid at 15 C. When heated again there was little evidence of melting until the temperature reached 25 to 30 C.

The lipid of *T. lipofera* was characterized by Kleinzeller (1944) as follows: carbon content of fatty acids 76 per cent; unsaponifiable material 6.8 to 8.8 per

cent of the lipid; mean molecular weight of the fatty acids, 268 to 280; and iodine value 55 to 90.

The lipids obtained from other yeasts have been found to contain fats, free fatty acids, phospholipids, and sterols (Bloor, 1943; Prescott and Dunn, 1940; Anderson, 1939). Daubney and Smedley-MacLean (1927) reported that the principal fatty acids in yeast lipid were the saturated palmitic and the unsaturated oleic and linoleic acids. There were also small amounts of the saturated lauric acid and possibly some arachidic acid. According to Anderson (1939), yeast lipid contains such saturated fatty acids as palmitic, stearic, lauric, butyric, arachidic, and tetracosanoic acids, and also unsaturated C_{16} and C_{18} acids. Palmitic and stearic acids were the principal fatty acids. Yeast lipid is characterized by a high proportion of unsaponifiable matter comprising one-third or more of the material extracted (Smedley-MacLean and Thomas, 1920). About one-half of the unsaponifiable material was sterol in combination with fatty acids. The sterol separated out from the rest of the lipid on standing (Smedley-MacLean, 1922). The phospholipids of *Saccharomyces cerevisiae* have been reported to comprise more than one-half the total ether-soluble material (Anderson, 1939).

The lipid extracted from *Penicillium javanicum* was reported by Ward, Lockwood, May, and Herrick (1935) to be similar to that obtained from higher plants and consisted of glycerides of palmitic, stearic, tetracosanoic, oleic, and linoleic acids, with a small amount of unsaponifiable matter. Bernhauer and Posselt (1937) found that the lipid obtained from *Aspergillus niger* contained 6 per cent glycerol and 68 per cent total fatty acids, three-fourths of which were made up of unsaturated acids.

SUMMARY

The yeast was recovered from various soils and appears to be a common soil inhabitant. On plates of nitrogen-free, glucose agar seeded with soil and incubated for two weeks or more it produced large, spreading, slightly opaque colonies with the cells dispersed in a watery slime. On nitrogen-deficient media the cells were large and nearly spherical with no tendency to produce chains; most cells had only one small bud. Each cell contained a single large refractive fat globule, which nearly filled the cell. Old cells tended to shed their outer membranes. Spores were produced, but the number in each ascus was variable. The spores had a light amber color. Some strains produced up to 8 spores, but one strain produced 16 or more when cultivated on some media. Under certain conditions the spores were formed in a saclike bud which remained attached to the parent cell. The bud was at first filled with granular contents, which later changed to spores. No spores were formed in the parent cell. The metabolism of the yeast was oxidative and sugars were not fermented. Nitrate was not utilized and gelatin was not attacked. In solution media, growth was sedimentary with no pellicle formation, and relatively few cells remained suspended except in aerated media.

The characteristics of the yeast are different from those of previously described yeasts which have been studied for lipid synthesis. It was not possible

to identify the yeast with known species. The yeast may be a new species or a yeast already described but with certain characteristics which have not been recognized up to this time.

Growth and lipid production were good in aerated glucose solutions containing small amounts of yeast extract as the nitrogen source. Under favorable conditions, 20 to 25 per cent of the consumed glucose was converted to yeast cells which contained 50 to 63 per cent lipid. From 10 to 14 per cent of the consumed glucose was recovered as lipid. The lipid content of the cells decreased as the nitrogen content of the medium increased. Only a small portion of the lipid could be extracted from the cells directly with fat solvents, but it became released by hydrolysis with dilute acid.

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