MORPHOLOGY OF *SACCHAROMYCES FRAGILIS* AND *RHODOTORULA GRACILIS* YEASTS GROWN IN COTTAGE CHEESE WHEY

By

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Bachelor of Arts

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1970

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
July, 1973

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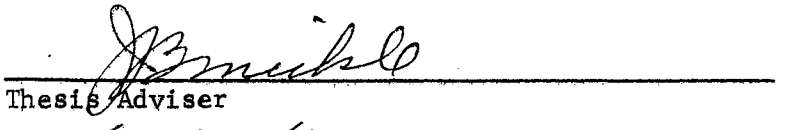
Thesis Approved:



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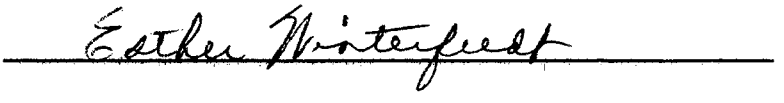

Dean of the Graduate College

ACKNOWLEDGMENTS

I want to express my appreciation particularly to Dr. James B. Mickle whose continued understanding, interest, and patient guidance throughout my research experiences made them truly worthwhile endeavors; to Mrs. Wanda Smith for growing the yeasts for these pictures and, at the same time, showing me how proper research is conducted; to Mrs. Olive Pryor for orienting me to the laboratory and the work; and to everyone on the first and second floors of the Dairy Building for helping me to maintain my equilibrium during the past two years.

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CHAPTER I

INTRODUCTION

The disposal of cottage cheese whey is a major problem for the cheese industry since this material has a relatively high biological oxygen demand (BOD), and many cities will not allow this material to be dumped into their sewers. Over 60% of the BOD in cottage cheese whey comes from lactose (milk sugar). Thus, removing the lactose prior to disposing of the whey removes a major portion of the organic material and simplifies the disposal problem (1).

Previous work in the Oklahoma State University Dairy Foods Research Laboratory had shown that two yeast species, Saccharomyces fragilis (NRRL Y-1156) and an adapted strain of Rhodotorula gracilis (NRRL Y-1091), would use lactose as a growth material, removing it from the whey, and reducing the BOD by 60% or more during the process (11, 16).

Knight (11) states that Porges, et al., showed that S. fragilis removed lactose more efficiently than any of the other yeast species he studied. Thus, in connection with lactose utilization, more work has been done with this species. Rh. gracilis was chosen because it is considered to be one of the most efficient fat-producing yeasts, often yielding 50-60% of its weight in lipid material (2, 14, and 20).

In connection with this earlier work, it was of interest to know when these yeast cells reached their maximum size since larger cells

are easier to separate from whey. An additional consideration in the case of Rh. gracilis was when a high fat content could be obtained; the higher the fat content in the microorganism, the more energy it contains and the more value it would have as human or animal feedstuffs.

The cellular morphology of these species (S. fragilis and the unadapted strain of Rh. gracilis) had been studied and reviewed by Lodder (12, 13), but a search of literature failed to disclose pictures of these yeasts grown on cottage cheese whey; no studies on the mutant strain of Rh. gracilis could be found. Therefore, this study was designed to follow the changes in cellular morphology of both yeast species when grown on cottage cheese whey and to observe whether or not unusual morphological variations occurred during growth.

CHAPTER II

REVIEW OF LITERATURE

Yeast morphology has been reviewed recently in a book titled The Yeasts edited by J. Lodder (12, 13). Among the various factors capable of inducing changes in yeasts from what is considered "normal" cellular morphology are: temperature, nutrients, age, pH, and the presence or absence of certain chemicals such as fusel oils, sodium thioglycollate, and indole acetic acid (20).

Saccharomyces fragilis

Early research on yeast morphology described the genus Saccharomyces as having oval or elongated oval vegetative cells. Recent researchers have expanded on this by including round cells in their descriptions; the cells generally occurred in pairs or small clusters and reproduced vegetatively by multilateral budding. In some species a pseudomycelium could be formed; a true mycelium was never present (13, 19).

Jörgensen first isolated S. fragilis from kefyr in 1909. He named the species fragilis because of the fragile cell wall (12). Heinrici (9) further classified this species as a lactose-fermenting, bottom yeast.

Lodder (12, 13) categorized S. fragilis according to its size and appearance in liquid and on solid media. Three days growth in malt extract at 28° C. produced characteristic cells with dimensions of

(2.0 - 6.0)μ x (3.5 - 10.0)μ. A pseudomycelium was usually formed. Three days growth on malt agar produced similar cells that were slightly longer and wider than those in the malt extract. The agar cultures were cream to brownish-cream colored. When ethanol was used as a sole carbon source, relatively weak growth resulted.

Recently, S. fragilis has been reclassified as a synonym of Kluyveromyces fragilis (Jörgensen) Van Der Walt. To account for this, Lodder (13) noted that ascospores in the species were bean-shaped instead of the characteristically round spores of the Saccharomyces. Asci (spore-containing sacs) formed by the species were fragile and ruptured easily at maturity while those of the Saccharomyces did not rupture. Additionally, this species was weakly fermentative and produced about 4 - 4.5% ethanol in media rich in sugar while the Saccharomyces are strongly fermentative (19).

Research of Saccharomyces cerevisiae, classed by Lodder (12) as the type species for the genus Saccharomyces, has indicated that a nitrogen limitation may cause abnormal cell elongation in continuous culture (5, 15); yet, this effect apparently has not been reported with S. fragilis.

Rhodotorula gracilis

Cryptococcus glutinis was first isolated by Fresenius from old starch paste. Several later researchers isolated red yeasts which they classified as Saccharomyces, Cryptococcus, or Torula glutinis. Because such a variety of yeasts were grouped in these genera, Hansen finally drew a distinction between the spore-forming and non-spore-forming yeasts when he recognized the non-spore-formers as the cause of many

failures in brewing. These yeasts he called "Torula". Since that time, a variety of species have been described, but seldom in sufficient detail to make reidentification possible (9, 12).

Attempts have been made to systematize these yeasts beginning in 1916 when Will grouped them under the family Torulaceae. Several researchers have since offered various classifications and nomenclatures. Harrison found these earlier attempts confusing and unacceptable; in 1928 he proposed a system based on physiological characteristics which retained Torulaceae as the family name (9). He grouped all forms that produced a rudimentary mycelium in the genus Mycotorula. Of the remaining species, those that produced a red pigment were classed as Rhodotorula; yeasts that formed pigments other than red were named Chromotorula; the genus Torula contained those yeast species forming no pigment at all (8).

The genus Rhodotorula contains cells characterized by researchers as oval, elongated oval, and round which reproduce vegetatively by multilateral budding. A pseudomycelium and a true mycelium might be formed by some species (8, 13, 19, and 22). These yeasts often contained oil-filled or fat-filled vacuoles stainable with Sudan Black B (22). This effect was noted particularly by Thyagaragan and Naylor (24) when they cultured Rh. glutinis on a glucose, peptone, and yeast extract medium. Red or pink pigments were always described as being produced by this genus (8, 13, 19, and 22). Since some authors also included orange and salmon-colored to yellow pigmented yeasts in their classification, in 1934 Lodder placed all yeast species forming carotenoid pigments together by combining the genera Rhodotorula and Chromotorula (19, 22).

Although some authors are in doubt as to the extent of actual species differentiation within the genus Rhodotorula, others have described specific species they believed to exist (9).

Lodder (13) classified the species gracilis as a synonym of the variety Rhodotorula glutinis (Fres.) Harrison var. glutinis. After three days growth at 23° C. in malt extract, characteristically-shaped cells of Rh. glutinis var. glutinis were described with dimensions of (2.3 - 5.5)μ x (4.0 - 10.0)μ. Cells of some strains became more elongated with lengths reaching 12 - 16μ; widths increased up to 7μ. Cellular morphology of these yeasts grown on malt agar was similar to that of those grown in malt extract with the exception that the cells of a particular strain became somewhat longer. Harrison (8) observed that cells of this species grown for 84 days on wort and wort agar exhibited similar shapes which were smaller than the younger cells observed. Lodder (13) indicated that gradual changes in dimension made it impractical to use cellular size for varietal designation. Lactose was not assimilated by this species; the use of ethanol as the sole carbon source produced good growth (12).

As cells of Rh. gracilis aged, Ruinen and Deinema (21) and Harrison (8) observed a gradual thickening of the cell wall. Rose (20) attributed this process to all encapsulated yeast species.

Several microorganisms have been the focus of studies for their possible use for industrial production of fat. According to some authors, Rh. gracilis seems to hold the most promise because it can yield as much as 50 - 60% of its weight as fat (14, 20). The Wartime Research Organization began investigations on fat production of Rh. gracilis in 1941 in Sweden. The majority of this work was performed at

the Royal Institute of Technology in Stockholm (2, 14).

Lundin (14) described two separate phases during growth periods of Rh. gracilis. The first was a protein formation phase during which abundant amounts of sugar, nitrogen, and phosphorus should be supplied. Yeast cell numbers increased rapidly during this time. When most of the nitrogen in the medium had been utilized, the fattening period began. Large quantities of sugar were needed, but only small amounts of phosphorus and no nitrogenous materials were required. The fat content of the cells increased rapidly while cell growth slowed down. Lundin indicated the fattening phase began after approximately 12½ hours of growth. Other researchers have reported growth and fattening phases similar to those of Lundin (2, 23).

CHAPTER III

EXPERIMENTAL PROCEDURES

Yeast Species

Pure cultures of S. fragilis and Rh. gracilis, the two yeast species studied in this experiment, were routinely carried in this laboratory on "YM" agar slants composed of 2.5% agar, 1% dextrose, 0.5% peptone, 0.3% yeast extract, and 0.3% malt extract. S. fragilis is a lactose-fermenting yeast (12); however, the original strain of Rh. gracilis could neither ferment nor assimilate lactose (12). Thus, it became necessary to adapt it for our use. This was accomplished employing a technique used by Nielsen and Nilsson (17) to adapt these yeasts to the utilization of xylose. After eight successive transfers onto lactose agar slants (2.5% agar, 2% lactose, 1% peptone, and 0.1% yeast extract), Rh. gracilis was able to use lactose, and cottage cheese whey as a growth medium (16).

Preparation and Growth

To prepare the S. fragilis for growth trials, a loop of pure culture was transferred from an agar slant to a broth containing 2% lactose, 1% peptone, and 0.1% yeast extract. When the yeasts were in the log phase of growth, a 10% inoculum of this "starter" broth was added to whey for the actual growth trials to obtain the S. fragilis yeast cells needed for observation of morphological changes (11).

The Rh. gracilis yeasts also were prepared in the above manner. When sugar determinations using "Clinitest" tablets[1] indicated that the lactose in the whey had been exhausted by the yeasts, a 5% sucrose supplementation was added to the whey for fattening them. After this sucrose was utilized, 5% additional sucrose was again added (16).

The temperature for both species was maintained at 95° ± 5° F. (35° ± 3° C.) during the growth period (11, 16). The pH of the medium for the S. fragilis was 4.8; Rh. gracilis grew in a medium with a pH of 5.0 ± 1.0 (11, 16). The composition of the whey medium assayed 4.53% lactose, 0.48% lactic acid, 0.5% protein, 0.95% ash, and 0.05% fat.[2]

Samples of both species were drawn at regular intervals so photomicrographs and further analyses could be made. The sample was always withdrawn before sucrose was added to the Rh. gracilis growth medium (16).

Storage of Yeast Samples

Photomicrographs taken of fresh cells compared with frozen and thawed ones revealed that freezing and thawing the cells did not appreciably change cellular morphology. Thus, the samples which were taken periodically were frozen until photographed and analyzed.

Photomicrographs

Photomicrographs were taken of wet mounts from the thawed samples

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[1]Reagent tablets used for the copper reduction test to detect sugar in urine. Active ingredients are copper sulfate, caustic soda, sodium carbonate, and citric acid. "Clinitest" is a trademark of the Ames Company, a division of Miles Laboratories, Inc.

[2]Analyses calculated on a weight/weight basis.

on a 25 x 75 mm. glass microscope slide covered with an 18 mm. square #1½ glass cover slip. These pictures were taken with a 35 mm. "Exacta" camera using "Panatomic X" black and white roll film, ASA 32, DIN 16; the ASA and DIN numbers are indicators of emulsion or film speed (4). This camera was mounted on an "American Optical series 10 Microstar" microscope. Exposure times were five seconds; and both a neutral density and a Ziess green filter (VG 9) were used on the microscope with the oil immersion objective (100 X) giving a field diameter of 182 $\pm$ 3μ (7).

A 1:20 dilution of each sample was prepared for the wet mount using a blood-diluting pipette with distilled water and methylene blue stain as diluents.

Measurements

Yeast cells were measured from pictures made from the photomicrographs taken. To do this, a photomicrograph was taken of a stage micrometer, focusing on the 20μ calibration separations. From this picture, it was possible to determine the number of microns in a millimeter by spanning two of the 20μ calibrations with a pair of dividers and measuring this distance on a ruler divided into millimeters (3). This figure was then used to convert the length and width of the yeast cells and the field diameter of the microscope from a millimeter scale to the actual dimensions in microns (measured as outlined above; 1 mm. equals 2.54 $\pm$ 0.12μ in the illustrations).

Every yeast cell in the picture, excluding buds, that could be clearly discerned was measured. Average cell dimensions were calculated; in specific instances, standard error was also determined.

Additional Analyses

Methylene blue was used as a cell stain for cell counts, using a hemocytometer and blood-diluting pipettes, which were performed routinely on both S. fragilis and Rh. gracilis yeasts.

Selected samples of Rh. gracilis were stained with Sudan Black B to determine if fat was present in the intracellular bodies. A smear was prepared by mixing one loopful of the yeasts and whey medium with one loopful of distilled water on a clean 25 x 75 mm. glass microscope slide. This was air-dried and heat-fixed. The entire slide was then flooded with Sudan Black B (0.3 gm. Sudan Black B in 100 ml. 70% ethyl alcohol) and allowed to stand ten minutes. The slide was drained and blotted dry with bibulous paper (an absorbant paper). Each slide was dipped in xylene several times and blotted dry. The smear was then counter-stained approximately 5 seconds with 0.5% aqueous safranin, dipped in tap water to remove excess stain, blotted, and air-dried (6). These slides were examined under a microscope for evidence of lipid-filled intracellular bodies.

Since Knight (11) had previously shown a relationship between turbidity readings and actual microscopic cell counts, growth of both yeast species was followed in this manner. One ml. aliquots of the yeasts and medium were diluted with nine ml. of distilled water; absorbance readings were taken on a Beckman Model B Spectrophotometer at 500 m . pH measurements of all samples were monitored with a Beckman pH meter (11, 16).

Lactose-content determinations, following the picric acid method of Perry and Doan (18), were performed on selected samples of S. fragilis and Rh. gracilis. Certain samples of the Rh. gracilis yeasts

were also analyzed for protein using the Kjeldahl method and fat content according to a method adapted from Steinberg and Ordal (10, 16, and 23).

All analyses performed on the above samples are summarized in Tables I and II in Chapter IV.

CHAPTER IV

RESULTS AND DISCUSSION

Saccharomyces fragilis

Photomicrographs of S. fragilis indicated that cellular growth and size increases closely paralleled the growth curve plotted from cell count data (Figure 1). The morphology of the cells during the growth period was primarily oval to elongated ovals.

The dimensions of these organisms ranged from (4.0)μ x (3.3 - 4.0)μ at the beginning of the growth trial (Sample "A") to (1.8 - 6.1)μ x (1.8 - 3.0)μ in the final sample, excluding buds, with an average of (4.0 x 3.4)μ in Sample "A" to (3.8 x 2.4)μ in Sample "D". The cells reached their maximum size in about seven hours. At this time cell counts were 450 x 10^6 and cell sizes ranged from (2.4 - 7.3)μ x (1.8 - 3.0)μ, S.E.[1] = 0.74μ.

Maximum cell numbers were attained in approximately 25 hours (Table I); in previous trials, maximum numbers had been reached in 7 - 12 hours (16). These yeasts occurred as single or budded cells, and multilateral budding was observed (Figures 2, 3, 4, and 5).

The pictures of S. fragilis grown on cheese whey showed that their morphology and size were similar to cells previously described and

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[1]S.E. is the standard deviation of the means for measurements of length and width.

illustrated by researchers when these organisms were grown on other media (9, 12, 13, and 19).

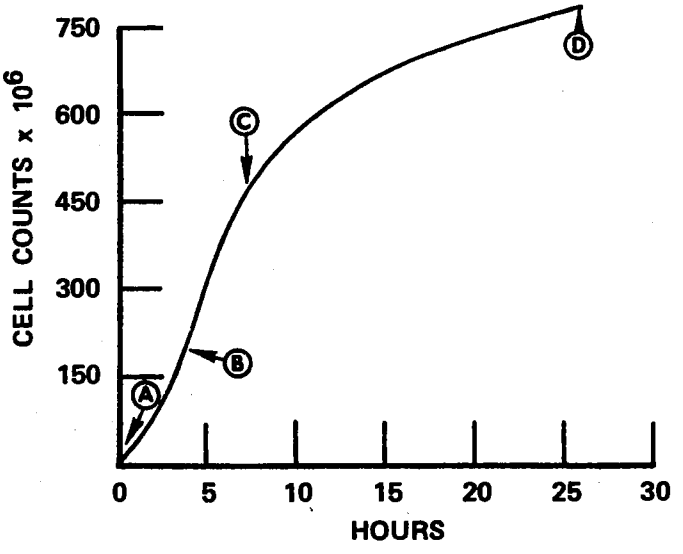

Figure 1. Cell Counts of S. fragilis Grown in Whey at 95° F. and pH 4.8; "A" - "D" Indicate Points Where Samples Were Drawn

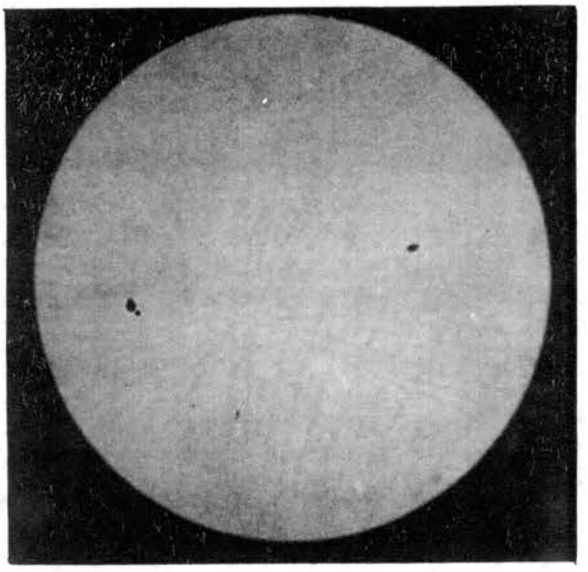

Figure 2. "A": S. fragilis at 0 Hours; Average Cell (4.0 x 3.7)μ.

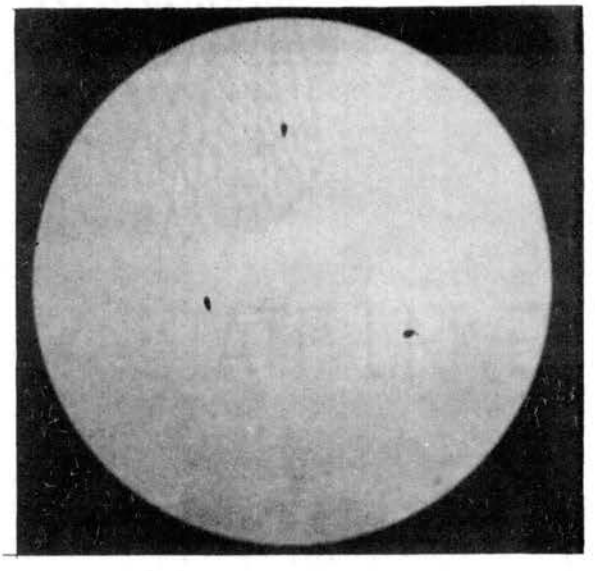

Figure 3. "B": S. fragilis at 4 Hours; Average Cell Size, (4.8 x 2.6)μ.

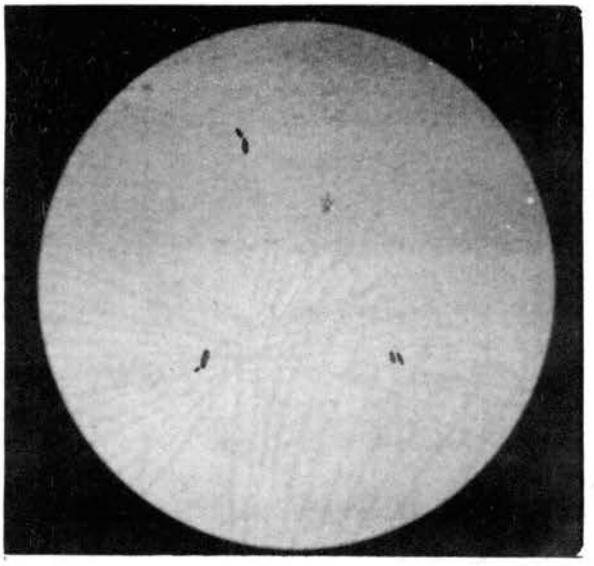

Figure 4. "C": S. fragilis at 7 Hours; Average Cell Size, (5.3 x 2.4)μ.

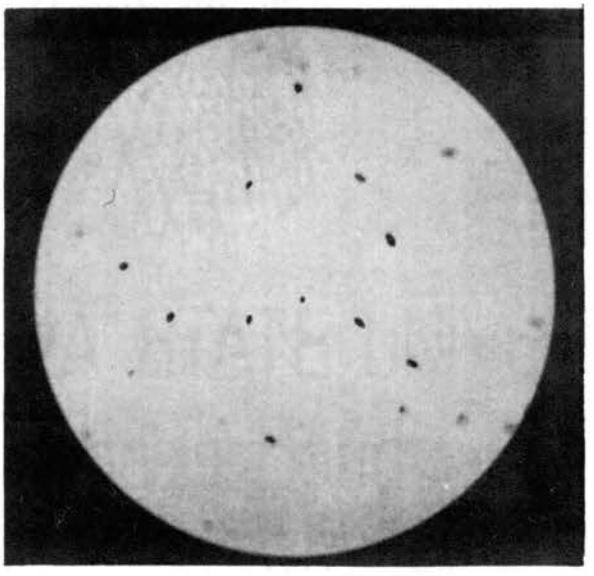

Figure 5. "D": S. fragilis at 25¼ Hours; Average Cell Size, (3.8 x 2.4)μ.

TABLE I

GROWTH OF S. FRAGILIS IN WHEY AS RELATED TO TURBIDITY AND SUGAR DETERMINATIONS

Hours Growth	Turbidity (Absorbance)	Cell Counts x 10^6	Sugar %[2] (Yeasts Plus Whey)
0.00	0.10	21	- -
4.00	0.33	195	- -
7.00	0.71	450	2.0
25.25	1.10	785	0.0

[2]Determinations made with "Clinitest" tablets which are reagent tablets used for the copper reduction test to detect sugar in urine. Active ingredients are copper sulfate, caustic soda, sodium carbonate, and citric acid. "Clinitest" is a trademark of the Ames Company, a division of Miles Laboratories, Inc.

Rhodotorula gracilis

The increase in size and number of Rh. gracilis yeasts closely followed the growth curve drawn from cell count data of this particular trial (Figure 6). Photomicrographs taken during the growth period showed both oval and elongated oval cells (Figures 7, 8, 9, and 10) which were typical shapes described by other investigators when these yeasts were grown on other media (8, 9, 12, 13, 19, 21, 22, and 24). Some of the cells were observed to have a cytoplasm which stained with methylene blue in an hourglass pattern similar to that found by Ruinen and Deinema (21).

Cell sizes ranged from (3.6 - 4.8)μ x (2.4)μ in Sample "A" to (4.8 - 9.7)μ x (3.0 - 6.1)μ in Sample "H". The average dimensions were (3.2 x 2.0)μ in Sample "A" and (7.0 x 4.4)μ in Sample "H".

The growth curve for Rh. gracilis (Figure 6) showed three maximum points in cell numbers. The first of these occurred after 18 hours growth (Point "B") when the organisms had used up most of the available protein in the original whey medium, which was 0.54% in Sample "A" and was reduced to 0.28% (Table II). The cell count at this time was 174 x 10^6; the cell sizes ranged from (3.6 - 9.7)μ x (2.4 - 4.8)μ, S.E. = 0.39μ. Average cell dimensions were (6.4 x 3.5)μ (Figure 7). Cell numbers reached a second maximum between 50 - 60 hours growth when the cell count was 325 x 10^6 (between Points "D" and "E" in Figure 6). After 66½ hours growth (Point "E"), all of the lactose in the whey was used up. At this time, 5% sucrose (weight/volume) was added to the medium (points marked "S" in Figure 6 refer to these 5% sucrose additions). Sucrose was used in preference to lactose or other sugars because it is commonly available commercially and is one of the least

expensive sugars on the market. Prior to this addition, the yeast cells appeared shorter and narrow (Figure 11); whereas, when they began growing again after the sucrose addition, they were longer with sizes ranging from (2.5 - 8.8)μ x (2.5 - 4.4)μ, S.E. = 0.46μ, and an average size of (6.4 x 3.0)μ (Figure 12). The cells continued to show fat-filled vacuoles. When Sample "G" (Figure 13) was taken immediately preceding the second 5% sucrose feeding, the third maximum point in cell numbers was attained; the cell count had increased to 635 x 10^6, and the cell sizes ranged from (3.6 - 9.1)μ x (2.4 - 5.5)μ, S.E. = 0.42μ. The cell dimensions averaged (6.4 x 4.2)μ. Six hours later, the cells had again started to grow (Sample "H", Figure 14); the range of cell dimensions was (4.8 - 9.7)μ x (3.0 - 6.1)μ, S.E. = 0.53μ. The cells had average dimensions of (7.0 x 4.4)μ.

With increasing age, the cell walls of the Rh. gracilis yeasts became progressively thicker, as noted by Ruinen and Deinema (21). This was especially noticeable in Figures 8, 10, and 13, taken after the yeasts had been growing 18, 42, and 90 hours respectively. Staining with Sudan Black B confirmed the presence of fat-containing vacuoles in the cells. The maximum fat production was noted in Sample "H" after the yeasts had been growing 96 hours. At this time, the yeast cells analyzed 28.41% fatty material.

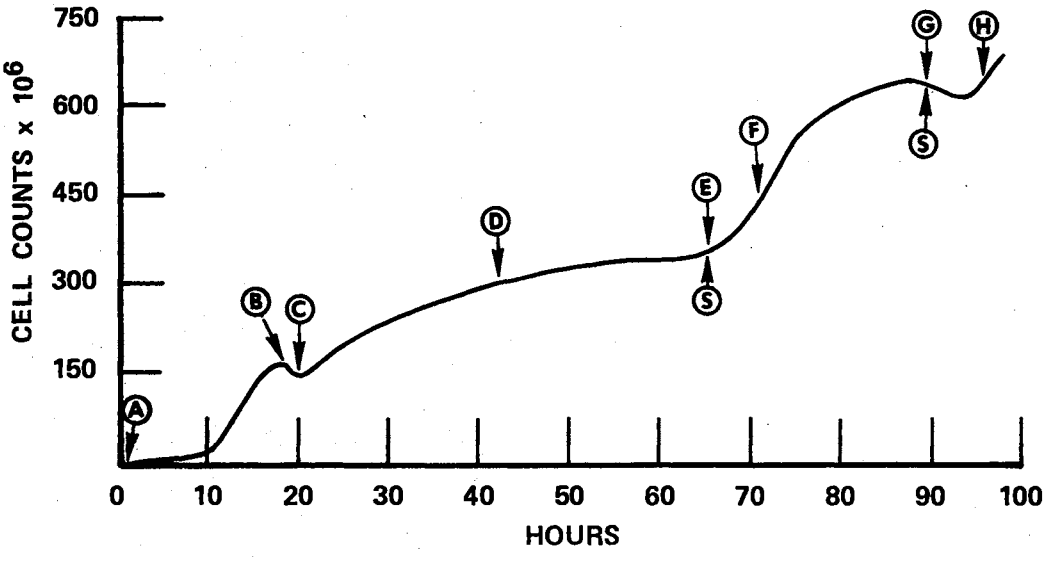

Figure 6. Cell Counts of Rh. gracilis Grown in Whey at 95° F. and pH 5.0; "A" - "H" Indicate Points Where Samples Were Drawn; "S" Indicates 5% Sucrose Additions after Sampling

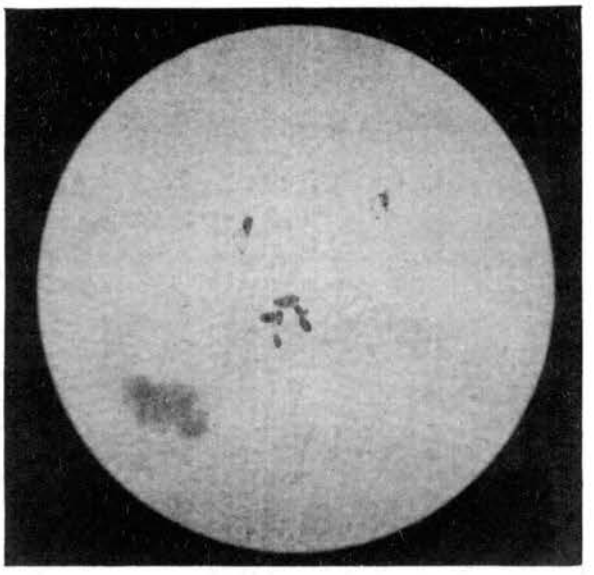

Figure 7. "A": Rh. gracilis at 0 Hours; Average Cell Size, (3.2 x 2.0)μ.

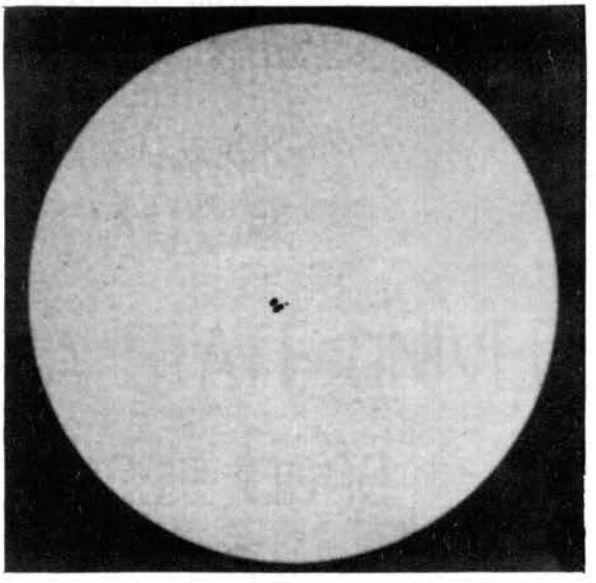

Figure 8. "B": Rh. gracilis at 18¼ Hours; Average Cell Size, (6.4 x 3.5)μ.

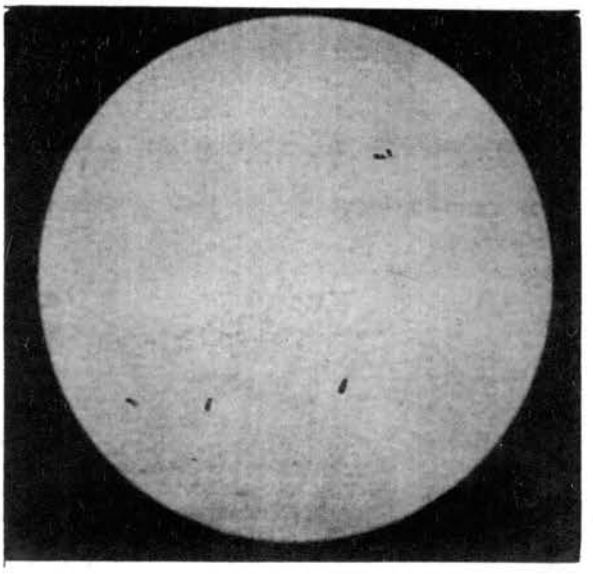

Figure 9. "C": Rh. gracilis at 20½ Hours; Average Cell Size, (4.7 x 2.0)μ.

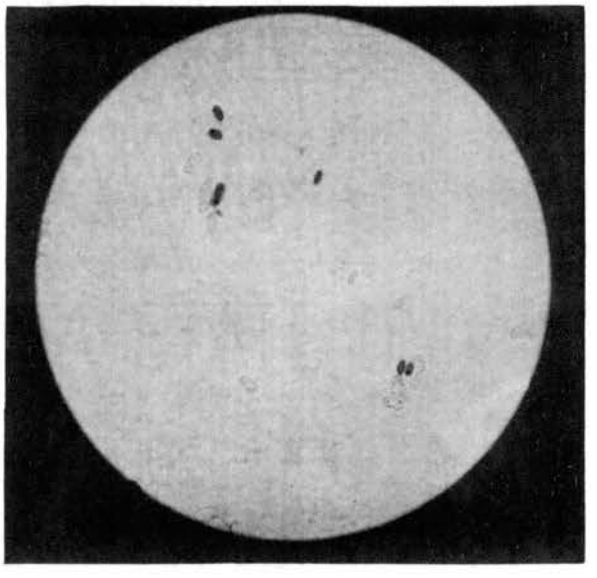

Figure 10. "D": Rh. gracilis at 42½ Hours; Average Cell Size, (6.5 x 3.8)μ.

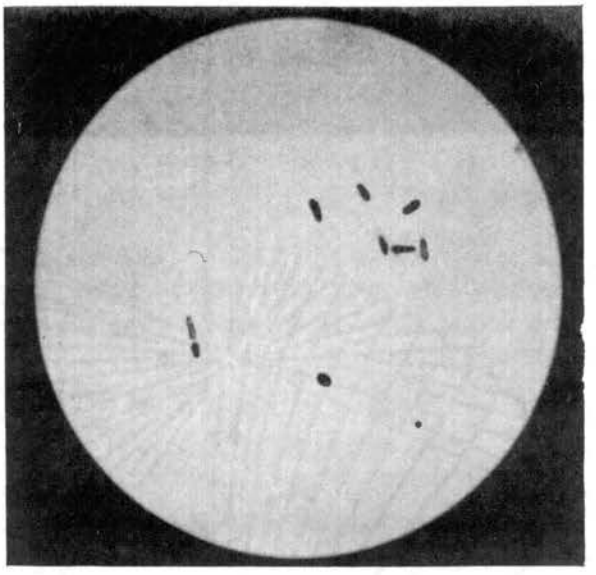

Figure 11. "E": Rh. gracilis at 66¼ Hours; Average Cell Size, (5.2 x 3.0)μ.

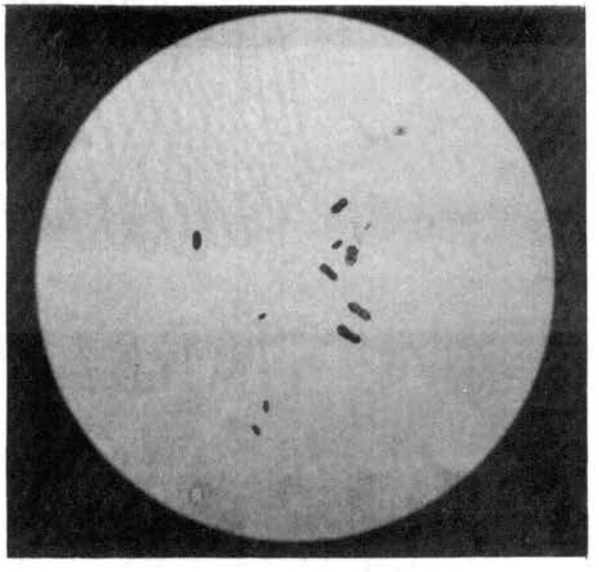

Figure 12. "F": Rh. gracilis at 71 Hours; Average Cell Size, (6.4 x 3.0)μ.

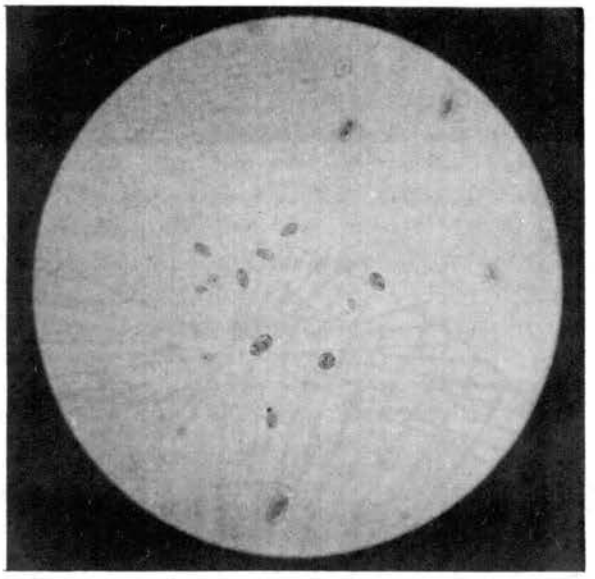

Figure 13. "G": Rh. gracilis at 90¼ Hours; Average Cell Size, (6.4 x 4.2)μ.

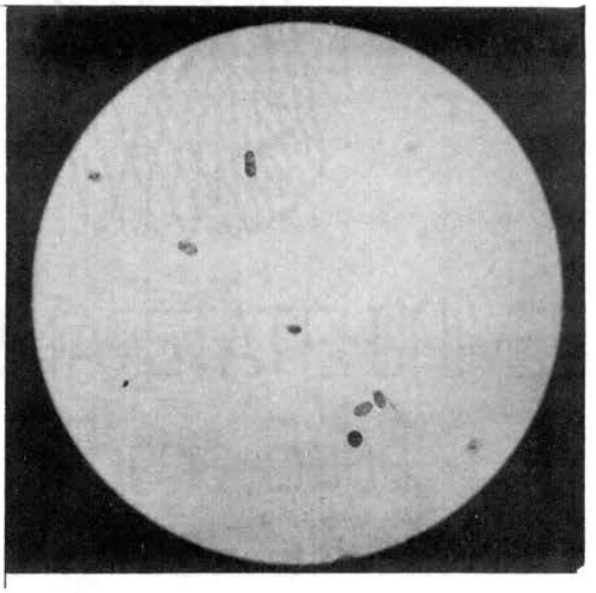

Figure 14. "H": Rh. gracilis at 96 Hours; Average Cell Size, (7.0 x 4.4)μ.

TABLE II

GROWTH OF RH. GRACILIS IN WHEY AS RELATED TO ANALYSES OF PROTEIN, LACTOSE, AND FAT AND SUGAR DETERMINATIONS

Hours Growth	Turbidity (Absorbance)	Cell Counts x 10^6	Protein %[3] (Whey)	Lactose %[3] (Whey)	Fat %[3] (Yeasts)	Sugar %[4] (Yeasts Plus Whey)
0.00	0.09	4	.54	5.31	- -	--
18.25	0.45	174	- -	5.40	- -	2+
20.25	0.49	165	- -	--	- -	2+
42.25	0.72	250	- -	4.82	- -	2
66.25[5]	1.00	345	.28	0.43	15.30	0
71.00	--	378	- -	--	- -	--
90.25[5]	1.39	635	- -	0.72	21.85	0
96.00	1.42	665	- -	7.67	28.41	2+

[3]Analyses calculated on a weight/weight basis.

[4]Determinations performed using "Clinitest" tablets.

[5]A 5% sucrose supplementation was added to the whey after the sample was taken.

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this study was to determine how the cellular morphology of Saccharomyces fragilis (NRRL Y-1156) and a strain of Rhodotorula gracilis (NRRL Y-1091), adapted to lactose utilization by repeated transfers on lactose agar, changed when grown in cottage cheese whey, if changes occurred that did not occur when these yeast species were grown in more standard growth media, and when maximum cell size and cell numbers were attained.

Previous work from this laboratory had shown that these yeasts can be used to remove the lactose from cottage cheese whey, thus reducing the biological oxygen demand (BOD) and making disposal of the whey effluent much simpler (11, 16). It was thought that the organisms could be harvested along with the protein remaining in the cheese whey and sold as human or animal feedstuffs. If so, it would be advantageous to harvest the organisms at the stage of maximum cell size and numbers.

Each yeast species was grown in cottage cheese whey under optimal conditions as had been previously determined in this laboratory (11, 16). Photomicrographs were taken of the cells sampled at regular intervals during the growth trial. These pictures indicated that the S. fragilis cells reached their maximum size in approximately seven hours and maximum cell numbers in about 25 hours. The average

dimensions of the S. fragilis yeasts ranged from (4.0 x 3.7) μ, in Sample "A" to (3.8 x 2.4) μ, in Sample "D".

The numbers of Rh. gracilis cells reached a first maximum when the available protein in the cheese whey medium was used up after 18 hours growth. Cells first began showing fat-filled vacuoles at this time. A second maximum in cell numbers was attained after growing approximately 50 - 60 hours. At 66¼ hours, when most of the lactose originally present in the whey was utilized, a 5% sucrose addition (w/v) was made to the medium. Cells continued to grow and show fat vacuoles. Cell numbers reached the third maximum when the first 5% sucrose supplementation was gone at the end of 90¼ hours. After a second 5% sucrose addition (w/v) at this time, the cells again started to increase in numbers and size. Cell walls of the adapted Rh. gracilis yeasts progressively thickened as the cells aged. The maximum fat production was found after the yeasts had been growing 96 hours; the cells were 28.14% fat. The range of average cell dimensions was (3.2 x 2.0) μ, in Sample "A" to (7.0 x 4.4) μ, in Sample "H".

The sizes and morphologies of the yeast cells observed were similar to those reported when the organisms had been grown on other media (8, 9, 12, 13, 19, 21, 22, and 24). However, this thesis is one of the most complete morphological studies available on the adapted strain of Rh. gracilis.

It appears that after seven hours increases in cell number of S. fragilis slow considerably and that most of the available lactose is utilized. Thus, in commercial operations, the yeasts could be removed from the medium at any time after this since the organisms have reduced the BOD of the whey 60% or more (11). Rh. gracilis, on the other hand,

does not appear to have much commercial application at this time since it requires a much longer time for growth and fat production. Perhaps future work will be able to shorten this growth time. Additionally, nutritional studies with small animals using harvested S. fragilis yeasts need to be carried out to determine the feasibility of utilizing the harvested cells as human or animal feed supplements.

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