Morphology of Two Yeast Species Grown In Cottage Cheese Whey

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The Story in Brief

The purpose of this study was to determine how the cellular morphology of *Saccharomyces fragilis* (NRRL Y-1156) and *Rhodotorula gracilis* (NRRL Y-1091) changed when grown in cottage cheese whey. Previous work from this laboratory had shown that these yeasts can be used to remove the lactose from cottage cheese whey, thus reducing the BOD (biological oxygen demand) and making disposal much simpler. It was thought that the organisms could be harvested along with the protein remaining in the cheese whey and sold as feed. If so, it would be advantageous to harvest the organisms at the stage of maximum cell size and number. These yeasts may be useful as a human food or an animal feed. In certain feeds, the amount of energy is of importance; thus, it was of interest to determine when these organisms reached a high fat content.

Each yeast species was grown in cottage cheese whey under optimum conditions as had been determined in this laboratory. Photomicrographs were taken of the cells at regular intervals during the growth trial. These pictures indicated that the *S. fragilis* cells reached their maximum size in approximately 7-12 hours and maximum cell numbers in approximately 25 hours. The dimensions of *S. fragilis* yeasts ranged from 3.8-5.3 x 2.4-3.7 u.

The numbers of *Rh. gracilis* cells reached a first maximum when the lactose in cheese whey was used up at approximately 66 hours. After adding 5 percent sucrose to the medium, the cells continued to grow and started to show fat vacuoles. The cell numbers reached a second maximum when the first 5 percent sugar addition was gone at the end of 90¼ hours. They reached a third maximum after a second addition of sucrose at the end of 96 hours. The cell walls of *Rh. gracilis* progressively thickened as the cells aged.

Although research of literature has failed to disclose pictures of either of these yeast species grown on cheese whey, the size and morphology was similar to that reported when the organisms had been grown on other media.

Introduction

The disposal of cottage cheese whey is a major problem for the industry since this material has a relatively high biological oxygen de-

mand and many cities will not allow this material to be dumped into their sewers. Over 60 percent 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.

Previous work in the OSU Dairy Foods Research Laboratory had shown that two yeast species, *Saccharomyces fragilis* and *Rhodotorula gracilis,* would use lactose as a growth material, thus removing it from the whey and reducing the BOD 60 percent or more during the process. In connection with this work, it was of interest to know when the cells reached their maximum size and, in the case of *Rh. gracilis* (a fat-producing yeast species), when a high fat content could be obtained. This information was needed because the larger cells are easier to separate from the whey and the higher the fat content in the organism, the more energy it contains and the more value it has for certain feed stuffs.

Although the cellular morphology of both of these species had been studied (2), research of literature failed to disclose pictures of these yeasts grown on cottage cheese whey.

Procedure

Pure cultures of *S. fragilis* and *Rh. gracilis,* the two yeast species studied in these experiments, were routinely carried on lactose-agar slants. *S. fragilis* is a lactose fermenting yeast; however, the original strain of *Rh. gracilis* could neither ferment nor assimilate lactose (2). Thus, it became necessary to adapt it for our use. After eight successive transfers on lactose-agar slants (a technique used by Neilsen and Nilsson (3) to adapt these yeasts to xylose), *Rh. gracilis* was adapted to the use of lactose (and whey) as a growth medium.

To prepare the *S. fragilis* for growth trials, a loop of pure culture was transferred from a slant to a broth containing 2 percent lactose, 1 percent peptone, and 0.1 percent yeast extract. After the yeasts exhibited rapid growth, a 10 percent innoculation 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 (1). The *Rh. gracilis* yeasts also were prepared in the above manner. When sugar determination indicated that the lactose in the whey had been exhausted by the *Rh. gracilis* yeasts, 5 percent sucrose was added to the whey for fattening them. After this sucrose was exhausted, 5 percent additional sucrose was again added. The temperature for both species was maintained at 95 ± 5° F (35± 3° C) during the growth period. Photomicrographs taken of fresh cells compared with frozen and thawed ones

had revealed that freezing and thawing the cells did not appreciably change cellular morphology. Thus, the samples which were taken at intervals were frozen until analyzed. Later, photomicrographs were taken of wet mounts from the thawed samples.

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. The methylene blue stain was routinely used as a cell stain, but selected samples of *Rh. gracilis* also were stained with Sudan Black B to determine if fat was present in the intracellular bodies.

Results and Discussion

Photomicrographs of *S. fragilis* indicated that cellular growth and size increases closely paralleled the growth curve (Figure 1). The morphology of the cells during the growth period was primarily ellipsoidal or cylindrical. The dimensions of these organisms ranged from 3.5-5.3 x 2.4-3.6 u excluding buds. The cells reached maximum size in about 7-12 hours and maximum cell numbers in approximately 25 hours. These yeasts usually 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 photographed by researchers when these organisms were grown on other media (2).

The increase in size of *Rh. gracilis* yeasts closely followed the growth curve (Figure 6) drawn from cell count data of this trial. During the growth period, photomicrographs showed both ellipsoidal and oval cells (Figures 7, 8, 9, and 10) which were typical shapes described by other investigators when the yeasts were grown on different media (2). Some of the cells were observed to have a cytoplasm which stained in an hourglass pattern similar to that found by Ruinen and Deinema (4).

There were three maximum points on this *Rh. gracilis* curve (Figure 6). The first occurred after 66 hours when the organisms had apparently used up most of the lactose and protein in the original whey media. At this time the cells had an average size of 5.2 x 3.0 u (Figure 11). Points marked "S" on the growth curve refer to the times at which 5 percent sucrose was added to the media. Prior to the addition of sucrose, the cells appeared short and narrow; whereas, when they started growing, they were longer with an average size of 6.4 x 3.0 u (Figure 12). At the time Sample "G" (Figure 13) was taken, just before the second sucrose feeding, cell numbers had increased to 635 x 10^6 and the average cell size was 6.4 x 4.2 u. Six hours later, when the cells had again started to grow (Sample "H", Figure 14), the average cell size was 7.0 x 4.4 u.

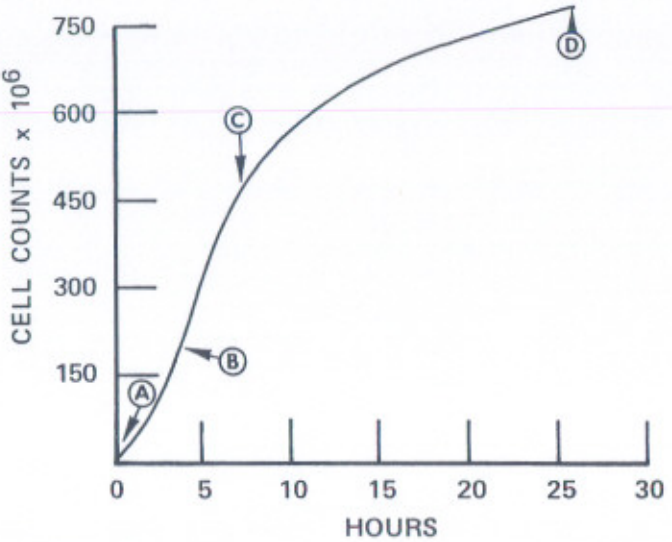

Figure 1. Cell Counts of *S. fragilis* Grown in Whey at 95°F and PH 4.8

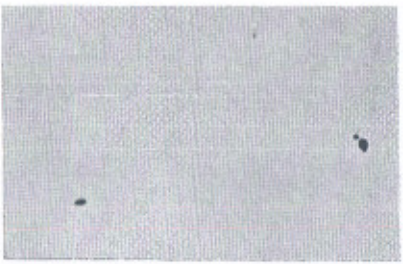

Figure 2. "A": *S. fragilis* at 0 Hours; Average Cell Size, 4.0 x 3.7 u

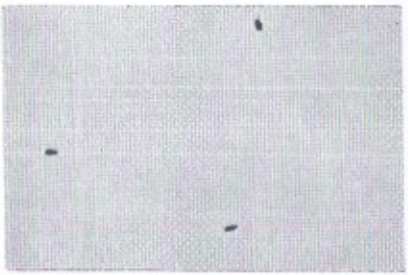

Figure 3. "B": *S. fragilis* at 4 Hours; Average Cell Size 4.8 x 2.6 u

Figure 4. "C": *S. fragilis* at 7 Hours; Average Cell Size 5.3 x 2.4 u

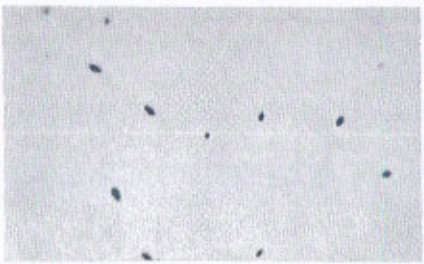

Figure 5. "D": *S. fragilis* at 25½ Hours; Average Cell Size 3.8 x 2.4 u

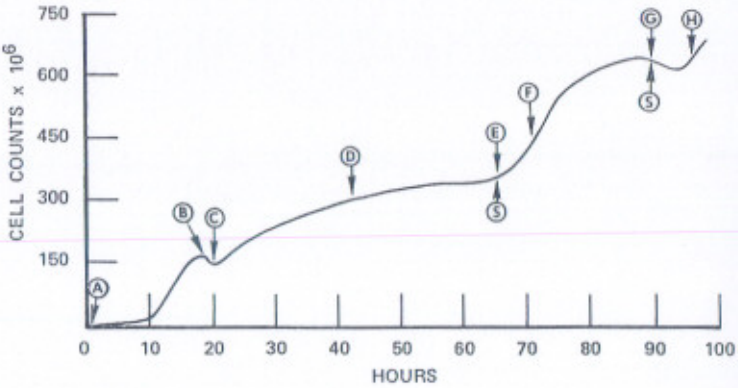

Figure 6. Cell Counts of *Rh. gracilis* Grown in Whey at 95°F and pH 5.0; "S" Indicates 5% Sucrose Feeding after Sampling

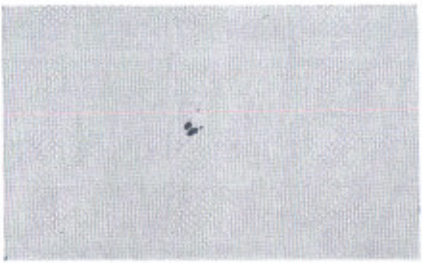

Figure 7. "A": *Rh. gracilis* at 0 Hours; Average Cell Size 3.2 x 2.0 u

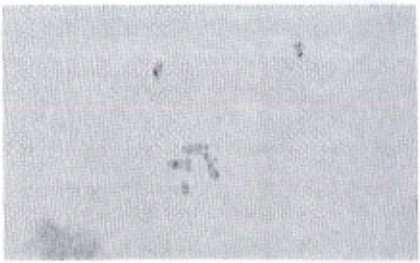

Figure 8. "B": Rh. *Gracilis* at 18¼ Hours; Average Cell Size 6.4 x 3.5 u

Figure 9. "C": *Rh. gracilis* at 20¼ Hours; Average Cell Size 4.7 x 2.0 u

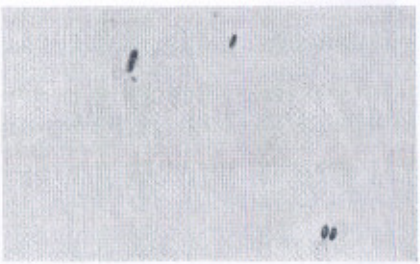

Figure 10. "D": *Rh. gracilis* at 42¼ Hours; Average Cell Size 6.5 x 3.8 u

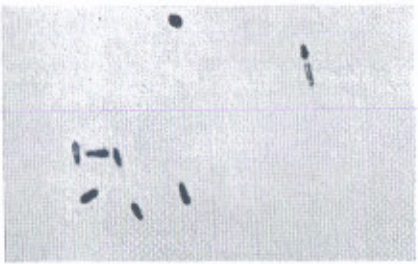

Figure 11. "E": *Rh. gracilis* at 66¼ Hours; Average Cell Size 5.2 x 3.0 u

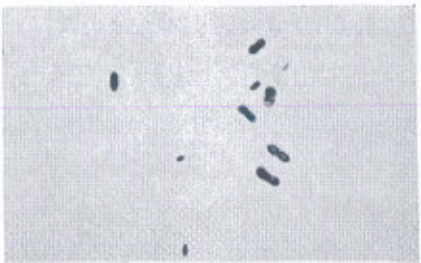

Figure 12. "F": *Rh. gracilis* at 71 Hours; Average Cell Size 6.4 x 3.0 u

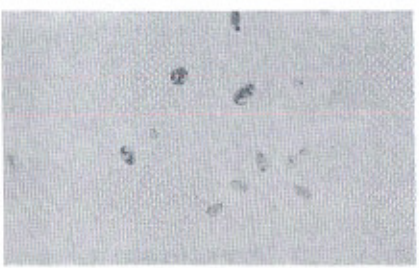

Figure 13. "G": *Rh. gracilis* at 90¼ Hours; Average Cell Size 6.4 x 4.2 u

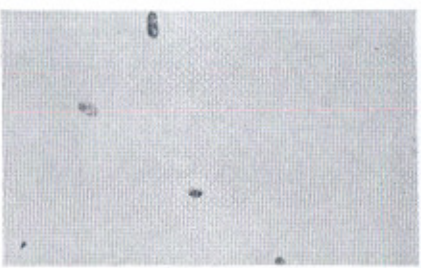

Figure 14. "H": *Rh. gracilis* at 96 Hours; Average Cell Size 7.0 x 4.4. u

With increasing age, the cell walls of the *Rh. gracilis* yeasts became progressively thicker as noted by other investigators (4). This was especially noticable 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.

Literature Cited

1. Knight, S., W. Smith, and J. B. Mickle. 1972. Cheese whey disposal using *Saccharomyces fragilis* yeast. Cultured Dairy Products Journal. 7: 17 (May, 1972).
2. Lodder, J. and N. J. W. Kreger-Van Rij. 1952. The Yeasts: A Taxonomic Study. John Wiley and Sons, New York. pp. 181-183, 647-649.
3. Nielsen, Niels and N. G. Nilsson. 1950. Investigations on respiration, growth, and fat production of *Rhodotorula gracilis* when cultivated in media containing different carbohydrates. Archives of Biochemistry. 25: 316.

4. Ruinen, J. and M. H. Deinema. 1968. Cellular and extracellular structure in *Cryptococcus laurentii* and *Rhodotorula* species. Canadian Journal of Microbiology. 14 (2) : 1133.

Other Related Projects

Changes in Postweaning feed Efficiency As a Result of Selection for increased Preweaning and Postweaning Growth Rate in Mice

M. A. Brown and R. R Frahm

Story in Brief

Growth performance to six weeks of age and feed efficiency between 21 and 42 days were determined for mice from three types of selection lines after 11 generations of selection. The three types of selection lines were: unselected control lines (CL), lines selected for increased weaning weight at 21-days of age (WWL) and lines selected for increased rate of gain between 21 and 42 days of age (ADGL). The WWL required more feed per unit of gain between 21 and 42 days of age than the CL, whereas the ADGL required less feed per unit of gain than the CL.

Both the WWL and ADGL significantly exceeded the CL in 21-day weight, 42-day weight, 21-42 day average gain and 21-42 day average daily feed consumption. The WWL were heavier at 21 days than the ADGL, but were lighter at 42 days of age, and gained slower from 21-42 days of age. Although the ADGL consumed more feed per day, they had a sufficiently larger rate of gain from 21-42 days of age that they required 1.3 grams less feed per gram of gain than did the WWL.