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 3.8-5.5 x 2.4-3.7 μ.

The numbers of R. 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 90½ hours. They reached a third maximum after a second addition of sucrose at the end of 96 hours. The cell walls of R. 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 dis-
posing 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, \textit{Saccharomyces fragilis} and \textit{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 \textit{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 sepa-
rate 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.

\textbf{Procedure}

Pure cultures of \textit{S. fragilis} and \textit{Rh. gracilis}, the two yeast species
studied in these experiments, were routinely carried on lactose-agar
slants. \textit{S. fragilis} is a lactose fermenting yeast; however, the original
strain of \textit{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 Nielsen and Nilsson
(3) to adapt these yeasts to xylose), \textit{Rh. gracilis} was adapted to the
use of lactose (and whey) as a growth medium.

To prepare the \textit{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 ex-
hibited rapid growth, a 10 percent inoculation of this “starter” broth
was added to whey for the actual growth trials to obtain the \textit{S. fragilis}
yeast cells needed for observation of morphological changes (1). The
\textit{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 \textit{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 main-
tained 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 μ 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 μ (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 μ (Figure 12). At the time Sample “G” (Figure 13) was taken, just before the second sucrose feeding, cell numbers had increased to 635 x 10⁶ and the average cell size was 6.4 x 4.2 μ. 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 μ.
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 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 25½ Hours; Average Cell Size 3.8 x 2.4 μ
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 μ

Figure 8. “B”: *Rh. Gracilis* at 18½ 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 0.5 x 3.8 μ

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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**


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.