

# NUTRITION, FEEDING, AND CALVES

## Effects of a *Saccharomyces cerevisiae* Culture on Ruminal Bacteria that Utilize Lactate and Digest Cellulose

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### ABSTRACT

The objective of this study was to determine the effects of a yeast (*Saccharomyces cerevisiae*) culture on lactate utilization and cellulose digestion by ruminal bacteria. Growth of *Selenomonas ruminantium* HD4 in medium that contained 5 g/L of DL-lactate, Trypticase, and yeast extract was stimulated 7 and 15% by 1 and 5% (vol/vol) yeast culture filtrate, respectively. The 1 and 5% yeast culture filtrate stimulated growth of *Selenomonas ruminantium* H18 and *Megasphaera elsdenii* B159 and T81 on 5 g/L of DL-lactate in medium without Trypticase or yeast extract. Growth of *Fibrobacter succinogenes* S85 and *Ruminococcus albus* B199 on 6 g/L of cellobiose was stimulated by the addition of yeast culture filtrate to medium without Trypticase or yeast extract. The yeast culture filtrate increased the concentrations of acetate and total volatile fatty acids that were produced by *Sel. ruminantium* HD4 and increased the concentrations of propionate and total volatile fatty acids that were produced by *Sel. ruminantium* H18 but did not alter end-product formation of *M. elsdenii* or cellulolytic bacteria. Treatment with yeast culture increased the initial rate but not the extent of cellulose digestion by *F. succinogenes* S85 and *Ruminococcus flavefaciens* FD1. Collectively, these results suggest that yeast culture provides soluble growth factors (i.e., organic acids, B vitamins, and amino acids) that stimulate growth of ruminal bacteria that utilize lactate and digest cellulose.

(**Key words:** *Saccharomyces cerevisiae* culture, lactate, cellulose, rumen)

### INTRODUCTION

For many years, ruminant nutritionists and microbiologists have been interested in manipulating the microbial ecosystem of the rumen to improve

production efficiency by domestic ruminants. Based on growing concern over the use of antibiotics and other growth promotants in the animal feed industry, interest in the effects of microbial feed additives on animal performance has increased during the past 5 to 10 yr. Addition of *Aspergillus oryzae* fermentation extracts and *Saccharomyces cerevisiae* cultures to ruminant diets has improved the digestibility of DM, CP, and hemicellulose; has increased ruminal bacterial numbers; has decreased ruminal lactate concentrations; and has increased milk production of cows in early lactation (6, 7, 28). However, response to fungal or yeast culture supplementation has been variable (13).

Compared with other widely used feed additives (e.g., ionophores), little research has been conducted to evaluate the effects of microbial feed additives on the growth and metabolism of predominant ruminal microorganisms. Our laboratory has shown that lactate utilization by the predominant ruminal bacterium *Selenomonas ruminantium* is stimulated by *A. oryzae* and *Sacc. cerevisiae* cultures (17, 18, 19, 27). In addition, *A. oryzae* stimulated lactate utilization by *Megasphaera elsdenii* (27). Both microbial feed additives appear to provide soluble growth factors (i.e., organic acids, B vitamins, and amino acids) that are required by both ruminal bacteria for growth on lactate (17, 18, 19, 27). Because several *A. oryzae* and *Sacc. cerevisiae* products are available and because all commercially available microbial feed additives cannot be assumed to have the same effect on ruminal bacteria, the objective of this study was to evaluate the effects of another yeast (*Sacc. cerevisiae*) culture (Diamond V XP; Diamond V Mills, Inc., Cedar Rapids, IA) on lactate utilization and cellulose digestion by several predominant ruminal bacteria.

### MATERIALS AND METHODS

#### Organisms and Growth Conditions for Lactate Studies

*Selenomonas ruminantium* strains HD4 and H18 and *M. elsdenii* strains B159 and T81 were used in this study. Strains HD4, B159, and T81 were ob-

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tained from J. B. Russell (Cornell University, Ithaca, NY), and strain H18 was obtained from H. J. Strobel (University of Kentucky, Lexington). The basal medium (pH 6.7) contained (per liter) 292 mg of  $K_2HPO_4$ , 240 mg of  $KH_2PO_4$ , 480 mg of NaCl, 480 mg of  $(NH_4)_2SO_4$ , 100 mg of  $MgSO_4 \cdot 7H_2O$ , 64 mg of  $CaCl_2 \cdot 2H_2O$ , 4000 mg of  $Na_2CO_3$ , 600 mg of cysteine-HCl, 1000 mg of Trypticase (BBL Microbiology Systems, Cockeysville, MD), 1 mg of resazurin, 500 mg of yeast extract (Difco Laboratories, Detroit, MI), 28.3 mmol of acetic acid, 8.1 mmol of propionic acid, 3.4 mmol of butyric acid, and 1 mmol each of valeric, isovaleric, isobutyric, and 2-methylbutyric acids (27). A similar medium was also used for growth studies in which Trypticase and yeast extract were omitted. Sodium DL-lactate (Sigma Chemical Co., St. Louis, MO) was prepared as a separate anaerobic solution (10%, vol/vol) under  $O_2$ -free  $CO_2$ ; the solution was autoclaved and added to the basal medium to achieve a final concentration of 5 g/L. Incubations were performed anaerobically under  $O_2$ -free  $CO_2$  at 39°C in batch culture.

#### Organisms and Growth Conditions for Cellulose Studies

*Fibrobacter succinogenes* S85, *Ruminococcus albus* B199, and *Ruminococcus flavefaciens* FD1 were used. *Fibrobacter succinogenes* S85 and *R. flavefaciens* FD1 were obtained from P. J. Weimer (USDA-ARS, Madison, WI). Strain B199 was obtained from H. J. Strobel (University of Kentucky). Basal medium with or without Trypticase and yeast extract was used as described previously. All incubations included 2% Schaefer's vitamin solution plus tetrahydrofolic acid, which contained (per liter) 20 mg of thiamine-HCl, 20 mg of Ca-D-pantothenate, 20 mg of nicotinamide, 20 mg of riboflavin, 20 mg of pyridoxine-HCl, 1 mg of *p*-aminobenzoic acid, 0.5 mg of biotin, 0.2 mg of vitamin B<sub>12</sub>, 0.125 mg of folic acid, and 0.125 mg of tetrahydrofolic acid. The vitamin mixture was prepared under an  $O_2$ -free  $N_2$  gas phase and filter-sterilized through a membrane filter (0.45- $\mu$ m pore size; Millipore Corp., Bedford, MA). Cellobiose was prepared in the same manner as DL-lactate, except under a  $N_2$  gas phase. The cellobiose was added to the medium to achieve a final concentration of 6 g/L. *Fibrobacter succinogenes* S85 and *R. flavefaciens* FD1 were also grown in a medium that contained (per liter) 900 mg of  $KH_2PO_4$ , 4000 mg of  $Na_2CO_3$ , 600 mg of cysteine-HCl, 1 mg of resazurin, 28.3 mmol of acetic acid, 8.1 mmol of propionic acid, 3.4 mmol of butyric acid, and 1 mmol each of valeric, isovaleric, isobutyric, and 2-methylbutyric acids. A mineral solution (80 ml) containing (per liter) 11.3 g of NaCl,

11.3 g of  $(NH_4)_2SO_4$ , 1.1 g of  $MgCl_2 \cdot 6H_2O$ , 0.8 g of  $CaCl_2 \cdot 2H_2O$ , 0.3 g of  $MnCl_2 \cdot 4H_2O$ , 0.3 g of  $FeSO_4 \cdot 7H_2O$ , 0.1 g of  $ZnCl_2$ , and 0.03 g of  $CoCl_2 \cdot 6H_2O$  was also added. This medium contained 3 g/L of Avicel® (FMC Corp., Philadelphia, PA) as the carbon and energy source.

#### Yeast Culture Preparation

The effects of Diamond V XP yeast (*Sacc. cerevisiae*) culture on the growth and fermentation end products of all five ruminal bacteria were examined. Because the yeast culture is bound to an insoluble carrier, a filter-sterilized filtrate was prepared by mixing 1 g of yeast culture in 50 ml of deionized water for 1 h at 25°C (17, 18). The slurry was then vacuum-filtered twice through a Whatman number 1 filter (Whatman Lab Sales, Hillsboro, OR), and the resulting filtrate was gassed overnight at 25°C with  $O_2$ -free  $CO_2$ . A serum bottle filled with  $CO_2$  was sealed with a butyl rubber stopper plus aluminum seal and autoclaved for 20 min. The filtrate was filter-sterilized through a membrane filter (pore size, 0.45  $\mu$ m) in an anaerobic glove box and injected into the sterile serum bottle filled with  $CO_2$ . A separate anaerobic filtrate was also prepared as previously described and was sterilized by autoclaving for 20 min.

#### Growth Studies

Roll tubes (18 × 150 mm) that were sealed with a butyl rubber stopper and that contained 9 ml of medium, yeast culture filtrate (1 or 5%, vol/vol) that had been autoclaved or filter-sterilized, and carbon source (lactate or cellobiose) were inoculated with 0.5 ml of a culture that had been grown for 24 h. The optical density at 600 nm was read with a spectrophotometer against a control sample of uninoculated medium. All incubations were performed anaerobically at 39°C and in duplicate. After 24 h, cells were removed from the medium by centrifugation (10,000 × *g* at 4°C for 15 min), and the supernatant was stored at -20°C until analyzed.

#### Uptake Assays

Uptake of D-glucose and L-lactate was measured in cells that had been grown in glucose or lactate medium and that were harvested (40 ml) anaerobically (under  $O_2$ -free  $CO_2$ ) during exponential growth (optical density at 600 nm, approximately 0.9) by centrifugation (10,000 × *g* at 4°C for 15 min) (17). Glucose was prepared and added to the growth medium as described by Martin (12). Cells were washed once with  $O_2$ -free 100 mM sodium potassium

phosphate buffer plus 5 mM MgCl<sub>2</sub> (pH 7.2) and resuspended in 10 ml of buffer. The 1.0-ml reaction mixture contained 100 mM sodium potassium phosphate buffer, 5 mM MgCl<sub>2</sub>, and 100 μl of whole cells. The effects of the yeast culture filtrate on uptake were evaluated by incorporating 10, 50, or 100 μl of filter-sterilized filtrate into the reaction mixture. The reaction was started by the addition of 1 mM D-glucose or L-lactate that contained 0.2 μCi of radio-label (298 mCi/mmol of D-[U-<sup>14</sup>C]glucose; DuPont/New England Nuclear Products, Boston, MA and 154 mCi/mmol of L-[U-<sup>14</sup>C]lactate sodium salt; Amersham Corp., Arlington Heights, IL). After incubation at 39°C for 5 min, the reaction was stopped by addition of 5 ml of 100 mM LiCl<sub>2</sub> to the reaction mixture. Cells were collected by filtration through a membrane filter (pore size, 0.45 μm). Filters were air-dried and counted by liquid scintillation. All incubations and assays were performed in duplicate.

### Analyses

The VFA in culture supernatants were measured by gas-liquid chromatography using a gas chromatograph (GC-14A Shimadzu; Shimadzu Scientific Instruments, Columbia, MO) (column temperature = 125°C, injector temperature = 170°C, and detector temperature = 175°C) equipped with an autosampler and GP 10% SP-1200/1% H<sub>3</sub>PO<sub>4</sub> 80/100 mesh size Chromosorb W AW column (Supelco, Bellefonte, PA) (26). Concentrations of VFA were corrected for the amounts of VFA present in the growth medium. Protein content of whole cells hydrolyzed with 0.2N NaOH (100°C for 15 min) was determined by the method of Lowry et al. (10), and the bovine serum albumin standard was treated similarly. Malate, formate, lactate, and succinate were quantitated by HPLC (Shimadzu LC-10A system controller, SIL-10A autosampler, C-R5A integrator, and RID-6A refractive index detector; Shimadzu Scientific Instruments; 50-μl loop, 50°C) with an organic acid column (Bio-Rad HPX-87H; Bio-Rad, Richmond, CA) (14, 22). Aspartate was analyzed by HPLC with a free amino acid analysis column (2). Glucose was determined using a coupled enzyme assay (1). Cellulose digestion was quantitated by measuring DM disappearance over time.

### Design

All experiments were performed in duplicate (n = 2) from two separate batch culture incubations. The growth data are presented graphically; means and

standard deviations are shown. Significance ( $P < 0.10$ ) between means was determined using Student's *t* test (25).

## RESULTS AND DISCUSSION

The addition of cereal grains to ruminant diets maximizes production levels in domestic ruminants. However, these cereal grains provide substrates for rapidly growing ruminal bacteria (i.e., *Streptococcus bovis*) that produce large quantities of lactate (24). Incorporation of some yeast cultures into ruminant diets is thought to help decrease lactate concentrations in the rumen by stimulating bacteria that ferment lactate, thus alleviating negative effects associated with lactic acidosis (4, 13, 29).

### Effects of Yeast Culture on Lactate Utilization

Growth of *Sel. ruminantium* HD4 in medium that contained Trypticase, yeast extract, and 5 g/L of DL-lactate was stimulated 7 and 15% at 24 h by 1 and 5% yeast culture filtrate, respectively (Figure 1A). Growth was stimulated ( $P < 0.05$ ) by 5% yeast culture filtrate after 6 h of incubation. No stimulation of growth was observed with the addition of yeast culture filtrate to *Sel. ruminantium* H18 cultures incubated in basal medium (Figure 1B), and addition of 1 or 5% yeast culture filtrate in the absence of DL-lactate resulted in no growth by either strain (Figure 1, A and B).

Trypticase is an enzymatic hydrolyzate of casein that contains peptides and branched-chain amino acids (21), and yeast extract is an excellent source of B vitamins and other unidentified growth factors (Difco Laboratories). To evaluate whether similar growth factors could be provided by the yeast culture filtrate, growth studies were performed in a minimal medium without Trypticase or yeast extract (Figure 1, C and D). Less growth occurred in the minimal medium with both HD4 and H18 over the 24-h incubation than in the basal medium (Figure 1, C and D vs. A and B). Compared with control incubations, 5% yeast culture filtrate stimulated growth of HD4 as much as 55%, but 1% yeast culture filtrate had little effect (Figure 1C). Both concentrations of filtrate were stimulatory to H18, and the 5% filtrate caused the greatest increase in growth (Figure 1D). Treatment effects were significant ( $P < 0.05$ ) after 6 h of incubation in minimal medium (Figure 1, C and D). Addition of 1 or 5% filtrate without lactate resulted in little growth of HD4 (Figure 1C); however, the 5% filtrate stimulated growth in H18 that was similar to growth observed in control tubes (Figure 1D).

Growth of *M. elsdenii* B159 on DL-lactate in basal medium was not stimulated by yeast culture filtrate (Figure 2A), but growth in minimal medium at 24 h was stimulated ( $P < 0.05$ ) 2.6- and over 4.4-fold by 1 and 5% yeast culture filtrate, respectively (Figure 2C). Treatment effects were significant ( $P < 0.05$ ) after 6 h of incubation. Growth of *M. elsdenii* T81 on lactate in basal medium was not affected by 1% yeast culture filtrate but was stimulated 10% by the 5% filtrate at 24 h (Figure 2B). Treatment effects were significant ( $P < 0.05$ ) after 10 h of incubation. In the absence of Trypticase and yeast extract, the growth of T81 at 24 h was stimulated by 1 and 5% yeast culture filtrate 4.3- and over 13-fold, respectively (Figure 2D). Treatment effects were observed after 4 h of incubation ( $P < 0.05$ ). Little growth of B159 with

either percentage of filtrate occurred in basal or minimal medium in the absence of DL-lactate (Figure 2, A and C), but the 5% filtrate supported some growth of T81 in the minimal medium (Figure 2D). As observed with both *Selenomonas* strains, less growth occurred in the minimal medium with both B159 and T81 over the 24-h incubation than in the basal medium (Figure 2, C and D vs. A and B). Collectively, these results suggest that the yeast culture filtrate provides growth factors that are similar to those found in Trypticase and yeast extract and that are required by *Sel. ruminantium* and *M. elsdenii* for growth on lactate. Similar results have been observed for *M. elsdenii* B159 in the presence of an *A. oryzae* fermentation extract (27).

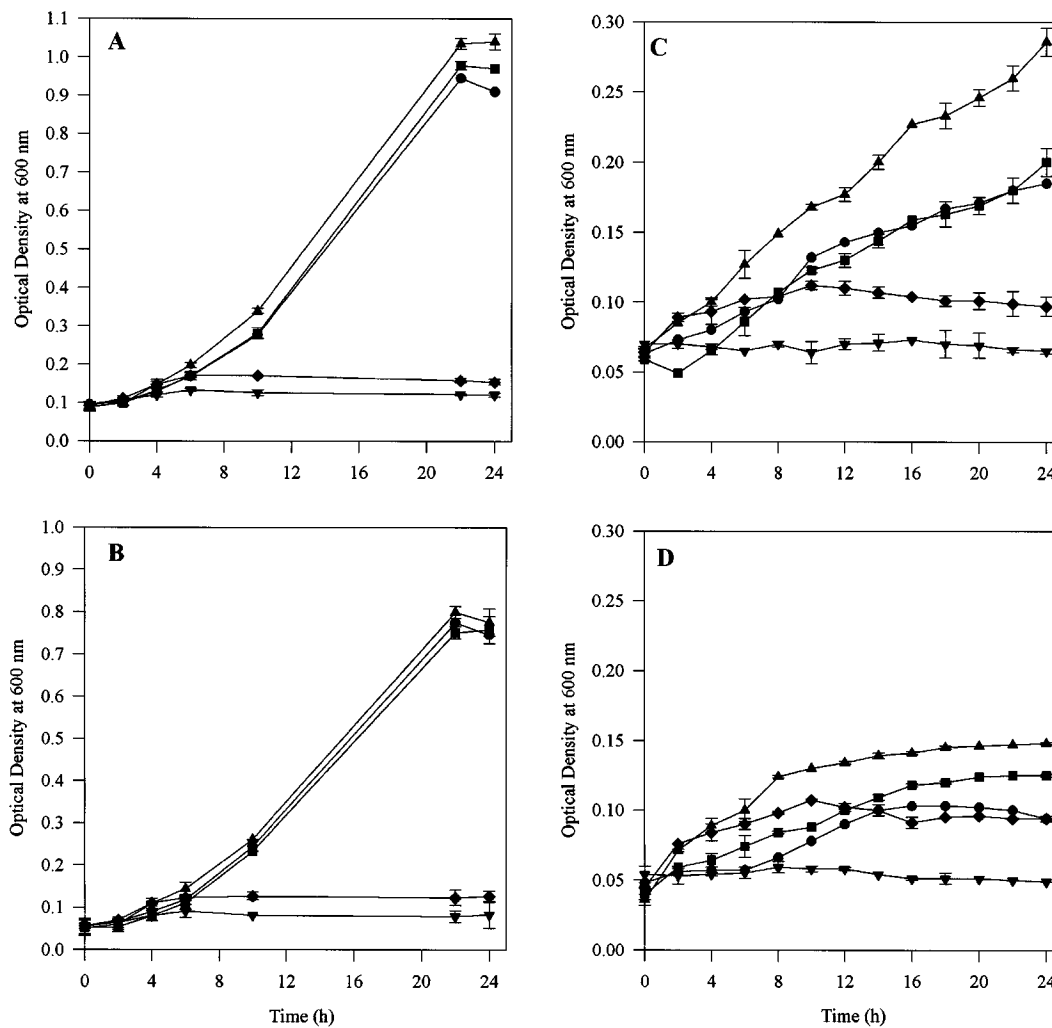


Figure 1. Effects of 1% (■) or 5% (▲) yeast culture filtrate that had been filter-sterilized on the growth of *Selenomonas ruminantium* HD4 (A and C) and H18 (B and D) on lactate in basal medium (A and B) and in medium without Trypticase and yeast extract (C and D). Control incubations (●) were performed in the absence of added yeast culture filtrate. Cells were also incubated in medium that contained only 1% (▼) or 5% (◆) yeast culture filtrate. Error bars represent standard deviation.

To determine the effects of yeast culture on lactate fermentation, bacteria that utilize lactate were grown in a basal medium that contained 5 g/L of DL-lactate and 1 or 5% yeast culture filtrate (Table 1). The addition of yeast culture filtrate caused some alterations in end-product formation. *Selenomonas ruminantium* produces primarily acetate and propionate when grown on lactate (8, 23). Addition of 5% yeast culture filtrate to *Sel. ruminantium* HD4 cultures increased ( $P < 0.05$ ) both acetate and total VFA concentrations, and propionate concentration tended to increase. With *Sel. ruminantium* H18, both 1 and 5% yeast culture filtrate increased ( $P < 0.05$ ) propio-

nate and total VFA concentrations; acetate increased numerically. The ratio of acetate to propionate did not change in either selenomonad culture.

*Megasphaera elsdenii* also produces acetate and propionate when grown on lactate, and some strains produce variable concentrations of butyrate and valerate (8, 11). Treatment with yeast culture had little effect on production of acetate or propionate by *M. elsdenii* B159 or T81 (Table 1). No changes in butyrate or valerate concentrations were detected (data not shown). A small decrease ( $P < 0.05$ ) in the ratio of acetate to propionate in B159 was associated with the addition of 5% yeast culture filtrate.

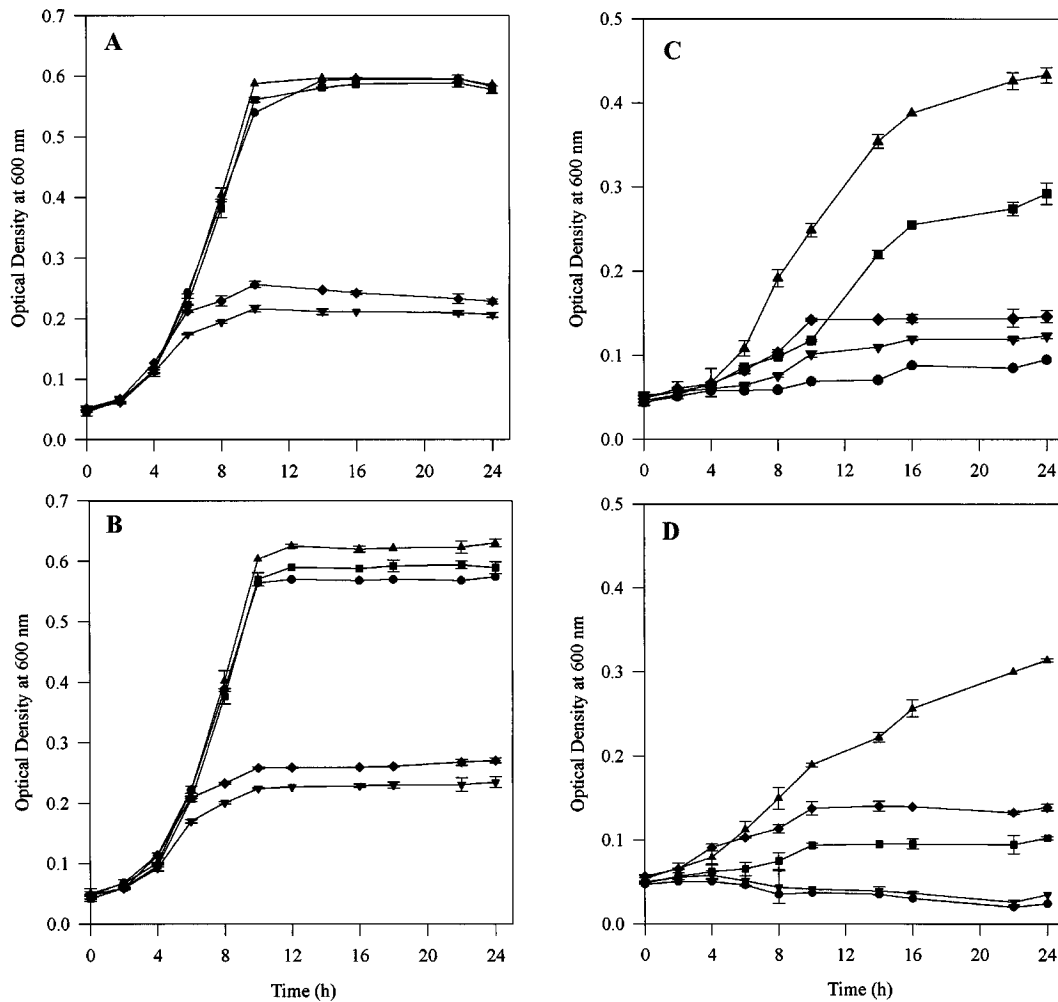


Figure 2. Effects of 1% (■) or 5% (▲) yeast culture filtrate that had been filter-sterilized on the growth of *Megasphaera elsdenii* B159 (A and C) and T81 (B and D) on lactate in basal medium (A and B) and in medium without Trypticase and yeast extract (C and D). Control incubations (●) were performed in the absence of added yeast culture filtrate. Cells were also incubated in medium that contained only 1% (▼) or 5% (◆) yeast culture filtrate. Error bars represent standard deviation.

TABLE 1. Effects of yeast culture filtrate on fermentation products produced by cells of *Selenomonas ruminantium* HD4 and H18 and *Megasphaera elsdenii* B159 and T81 grown on 5 g/L of lactate.

Strain and treatment	Fermentation product <sup>1</sup>							
	Acetate (A)		Propionate (P)		Total VFA		A:P	
	(mM)							
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
<b>HD4</b>								
Control	8.0	0.5	19.4	2.9	27.0	1.5	0.41	0.1
1% Yeast filtrate	10.5	1.4	23.0	1.5	32.9	2.7	0.46	0.03
5% Yeast filtrate	12.8*	0.04	25.6	0.8	37.4*	1.0	0.50	0.01
<b>H18</b>								
Control	11.0	0.9	15.3	1.6	26.7	2.0	0.71	0.03
1% Yeast filtrate	14.2	0.5	23.0*	0.1	37.0*	0.7	0.62	0.03
5% Yeast filtrate	11.8	3.1	22.9*	0.5	33.9*	2.1	0.51	0.1
<b>B159</b>								
Control	6.7	0.8	8.2	1.0	33.2	2.6	0.82	0
1% Yeast filtrate	5.5	0.1	8.0	0.7	30.8	0.4	0.69	0.05
5% Yeast filtrate	5.8	0.8	8.6	1.8	30.3	2.5	0.67*	0.05
<b>T81</b>								
Control	1.7	2.3	8.1	0.4	24.7	3.0	0.21	0.3
1% Yeast filtrate	0.1	0.6	7.0	0.2	16.2	8.0	0.02	0.1
5% Yeast filtrate	0.6	0.3	7.8	0.6	21.4	0.7	0.08	0.03

<sup>1</sup>Each value represents the mean of three determinations.

\*Means within a column for each bacterial strain differ from the control ( $P < 0.05$ ).

### Effects of Yeast Culture on the Uptake of Lactate and Glucose

Recent research (17, 18, 19, 27) has demonstrated that soluble components in *A. oryzae* and another *Sacc. cerevisiae* culture filtrate stimulated lactate uptake by *Sel. ruminantium* and *M. elsdenii*. Organic acids, amino acids, and B vitamins in both filtrates may play a role in stimulating lactate uptake by both bacteria (17, 18, 19, 27). To determine whether the Diamond V XP yeast culture filtrate also stimulated lactate uptake, assays were performed in the presence of yeast culture filtrate. Assays that were conducted to determine the uptake of L-lactate were performed numerous times, but we were unable to detect any stimulation of lactate uptake by the yeast culture filtrate in either *Sel. ruminantium* or *M. elsdenii* strains (data not shown). These results suggested that some factors in the filtrate may inhibit lactate uptake.

Because glucose is a preferred substrate and represses lactate utilization in *Sel. ruminantium* (20), the concentration of glucose in the yeast culture filtrate was determined (Table 2). Glucose was present in the filtrate, as were lactate, malate, formate, succinate, and aspartate. Even though *Sel. ruminantium* requires organic acids (e.g., aspartate or malate) to utilize lactate (9, 17), the glucose and lactate in the filtrate might have inhibited radio-

labeled L-lactate uptake during the short, 5-min assay. However, because lactate utilization is not catabolite-repressed in *M. elsdenii* (20), we cannot dismiss the theory that some other unidentified soluble components in the filtrate may also be involved. Although the yeast culture filtrate appeared to inhibit lactate uptake over the short-term (5 min), the same filtrate stimulated growth of both *Sel. ruminantium* and *M. elsdenii* on lactate over 24 h (Figures 1 and 2).

The effects of yeast culture filtrate on glucose uptake by *Sel. ruminantium* HD4, *M. elsdenii* B159, and *F. succinogenes* S85 were also evaluated (Table 3). In all three bacteria, glucose uptake tended to decrease as the amount of yeast culture filtrate increased. The

TABLE 2. Concentrations of carbon sources in yeast culture filtrate.

Carbon source	Concentration <sup>1</sup>	
	(mM)	
	$\bar{X}$	SD
Glucose	5.6	1.0
Lactate	4.3	1.5
Malate	6.0	0.9
Formate	1.6	0.7
Succinate	0.9	0.8
Aspartate	0.9	0.01

<sup>1</sup>Each value represents the mean of three determinations.

TABLE 3. Effect of yeast culture filtrate on glucose uptake by *Selenomonas ruminantium* HD4, *Megasphaera elsdenii* B159, and *Fibrobacter succinogenes* S85.

Treatment	Specific activity <sup>1,2</sup>					
	HD4		B159		S85	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Control	9.1	0.2	1.7	0.4	4.3	0.41
Yeast filtrate						
100 $\mu$ l	4.3*	0.7	0.8	0.2	3.6	0.35
50 $\mu$ l	4.9	1.0	0.8	0.2	4.1	0.65
10 $\mu$ l	8.9	0.2	1.6	0.5	4.9	0.14

<sup>1</sup>Expressed as nanomoles of glucose uptake per milligram of protein per minute.

<sup>2</sup>Each value represents the mean of two determinations.

\*Mean within a column differs from the control ( $P < 0.10$ ).

highest amount of filtrate inhibited ( $P < 0.10$ ) glucose uptake 53% in HD4. Some of this inhibition might have been due to the glucose that was present in the filtrate (Table 2), but other factors might also have been involved.

#### Effects of Yeast Culture on Cellulose Disappearance

Previous research (5, 16, 28) has shown that treatment with yeast culture increases the number of cellulolytic bacteria in the rumen and, in some cases, increases cellulose degradation. Newbold et al. (15, 16) suggested that *A. oryzae* and *Sacc. cerevisiae* stimulated the rate rather than the extent of fiber digestion by ruminal microorganisms. When *F. succinogenes* S85 and *R. flavefaciens* FD1 were incubated with cellulose, the 5% yeast culture filtrate increased ( $P < 0.05$ ) cellulose disappearance as much as 11% in both cultures after 24 h of incubation (Figure 3). No change in cellulose disappearance was found after 48 or 72 h. These results suggest that the yeast culture filtrate stimulates the initial rate of cellulose degradation by these two predominant cellulolytic bacteria without influencing the extent of degradation. Previous studies (4, 29) have reported that the stimulation of cellulose degradation by yeast culture is associated with a decreased lag time, which results in increased initial rates of digestion but not in increased extent of digestion by ruminal microorganisms.

#### Effects of Yeast Culture on Cellobiose Fermentation

Because cellulolytic ruminal bacteria utilize the end products of cellulose digestion (i.e., cellobiose and glucose) for growth, the effects of yeast culture

filtrate on the growth of *F. succinogenes* S85, *R. flavefaciens* FD1, and *R. albus* B199 on cellobiose were determined (Figure 4). Growth of *F. succinogenes* S85 on 6 g/L of cellobiose in basal medium

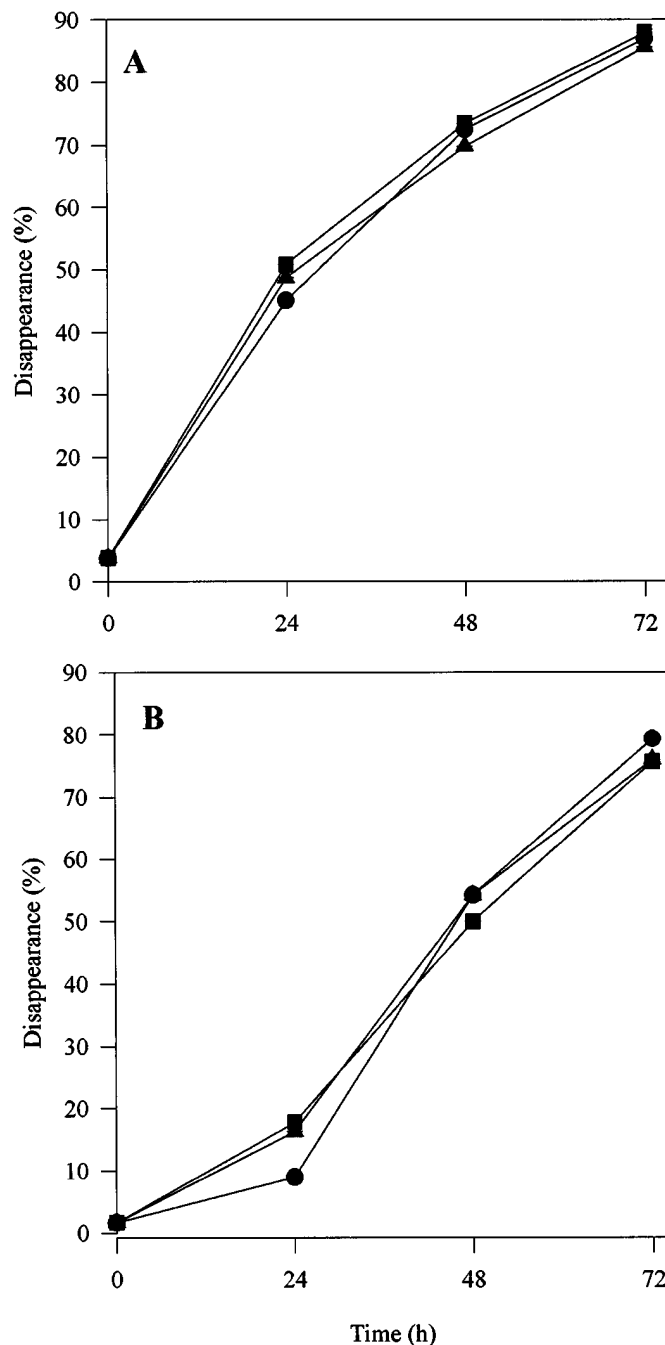


Figure 3. Effect of 1% (■) or 5% (▲) yeast culture filtrate that had been filter-sterilized on cellulose digestion by *Fibrobacter succinogenes* S85 (A) and *Ruminococcus flavefaciens* FD1 (B). Control incubations (●) were performed in the absence of added yeast culture. Error bars representing standard deviation are smaller than data point symbols.

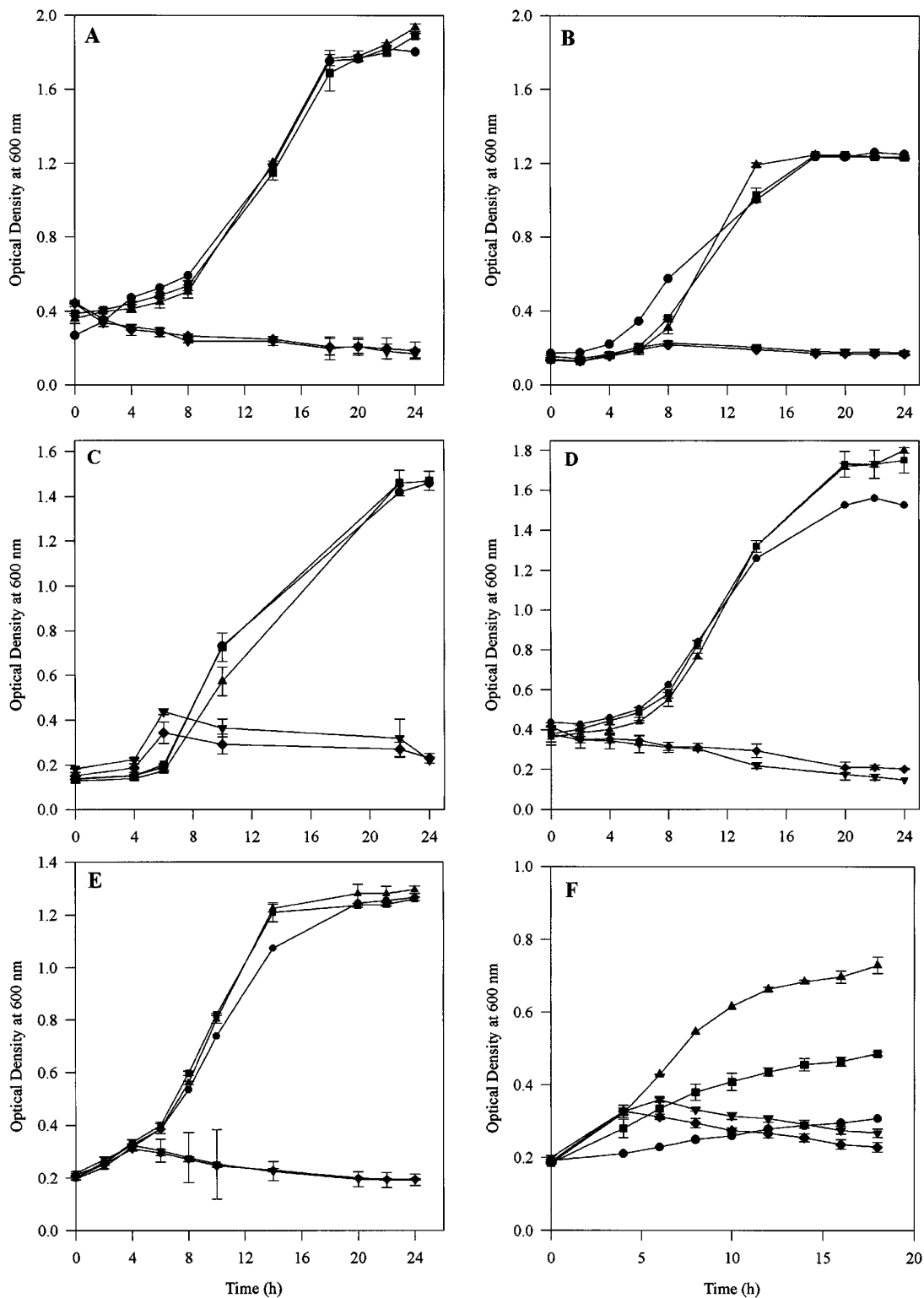


Figure 4. Effects of 1% (■) or 5% (▲) yeast culture filtrate that had been filter-sterilized on the growth of *Fibrobacter succinogenes* S85 (A and D), *Ruminococcus flavefaciens* FD1 (B and E), and *Ruminococcus albus* B199 (C and F) on cellobiose in basal medium (A, B, and C) and in medium without Trypticase and yeast extract (D, E, and F). Control incubations (●) were performed in the absence of added yeast culture filtrate. Cells were also incubated in medium that contained only 1% (▼) or 5% (◆) yeast culture filtrate. Error bars represent standard deviation.

was slightly stimulated (7%) by 5% yeast culture filtrate, but treatment effects were not significant until 24 h ( $P < 0.05$ ; Figure 4A). *Ruminococcus flavefaciens* FD1 and *R. albus* B199 were not stimulated by the addition of yeast culture filtrate to the basal medium (Figure 4, B and C). Addition of 1 or 5% yeast culture filtrate to medium without Trypticase or yeast extract stimulated growth of S85 15 and 18%, respectively; treatment effects were significant ( $P < 0.05$ ) after 20 h (Figure 4D). Yeast culture treatment did not stimulate growth of FD1 in minimal medium (Figure 4E), but both filtrate percentages increased ( $P < 0.05$ ) the growth of B199 (Figure 4F). When the three bacteria were incubated separately in basal medium in the absence of cellobiose, no growth occurred on the yeast culture filtrate (Figure 4, A, B, and C). However, some growth occurred in the presence of either filtrate concentration with B199 after 4 to 6 h in minimal medium (Figure 4F). No changes in end-product formation associated with yeast culture supplementation of the basal medium was seen for any of the cellulolytic bacteria (data not shown).

In addition to the yeast culture prepared at 1 g/50 ml, similar studies were also performed with yeast culture prepared at 2.5 and 5 g/50 ml. Results from those studies are not shown, but responses were similar to those observed with the yeast culture prepared at 1 g/50 ml. Dawson et al. (5) reported that heat-inactivated yeast culture preparations have no effect on ruminal bacterial growth, suggesting that live yeast cells are necessary for the stimulation of growth. Preparations of the Diamond V XP yeast culture filtrate that were autoclaved stimulated growth in a manner similar to that of yeast culture filtrate that had been filter-sterilized as was reported previously (data not shown). These results suggest that some stimulatory factors in the yeast culture filtrate are not inactivated by heat. The yeast culture filtrate does contain malate (Table 2), and malate stimulates growth on lactate, as well as lactate uptake by *Sel. ruminantium*, and can be autoclaved (17, 18, 19).

### CONCLUSIONS

Growth of several ruminal bacteria in a minimal medium without Trypticase or yeast extract was stimulated in the presence of yeast culture filtrate. Based on the known growth requirements of these bacteria, these results suggest that soluble factors (i.e., B vitamins, amino acids, and organic acids) in the filtrate are involved in stimulating growth. Or-

ganic acids (fumarate and malate) and aspartate are utilized fairly quickly (10 to 24 h) by mixed ruminal bacteria (3, 22). In addition, microbial synthesis of B vitamins in the rumen is presumed to satisfy both the microbial and animal requirements for these nutrients (30). However, to our knowledge, it is unknown whether microbial synthesis of B vitamins is constant across diets. Because these soluble factors, particularly B vitamins and organic acids, may not be present within the rumen throughout the entire feeding cycle, dietary supplementation with yeast cultures that are high in these growth factors may improve ruminal fermentation. Not all *Sacc. cerevisiae* cultures effectively modify the ruminal bacterial population (16). Understanding how various *Sacc. cerevisiae* cultures affect the growth and metabolism of important ruminal bacteria might eventually lead to the development of microbial feed additives that are specific to the diet.

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