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NOTES

Utilization of Nucleic Acids by *Selenomonas ruminantium* and Other Ruminal Bacteria

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Species of ruminal bacteria were screened for the ability to grow in media containing RNA or DNA as the energy source. *Bacteroides ruminicola* D31d and *Selenomonas ruminantium* HD4, GA192, and D effectively used RNA for growth, but not DNA. *B. ruminicola* D31d was able grow on nucleosides but not on bases or ribose. The *S. ruminantium* strains were able to grow when provided with either nucleosides or ribose but not bases. Strains of *S. ruminantium*, but not *B. ruminicola* D31d, were also able to use nucleosides as nitrogen sources. These data suggest that RNA fermentation may be a general characteristic of *S. ruminantium*.

Ruminants subsist on diets of plant material composed largely of complex carbohydrates such as cellulose, hemicellulose, starch, and pectin, and these polysaccharides provide the resident ruminal microbiota with the majority of energy for their metabolism. Plants also contain other materials such as nucleic acids that, although quantitatively not as abundant as plant polysaccharides, can be a potential source of energy and cell precursors for microbial growth. The nucleic acid content of feedstuffs varies considerably with source, ranging from about 5% (3) in legumes such as alfalfa to 0.24% in flaked corn (23). Additional sources of DNA and RNA would include sloughed ruminal epithelia and lysed microbial cells (16).

The fate of nucleic acids in the rumen is short-lived, indicating extensive degradation by ruminal microorganisms (17, 21, 23). The utilization of nucleic acids by ciliate protozoa (primarily from engulfed bacteria) has long been recognized (4), but information on the use of nucleic acids by ruminal bacteria is scarce. In a few cases the effect of added nucleic acids, nucleotides, or bases has been examined to see whether these might be stimulatory or required for growth by some species of ruminal bacteria (7, 13, 19, 20). However, the ability of ruminal bacteria to utilize nucleic acids as an energy source has not been reported. An objective of the current study was to determine which ruminal bacteria could use RNA or DNA as an energy source.

The organisms used in this study were obtained from our culture collection (Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Ill.). All cultures were grown anaerobically under an 80% nitrogen–20% carbon dioxide atmosphere in batch cultures at 39°C and pH 6.8. In experiments examining the use of DNA, RNA, nucleosides, bases, or sugars as energy source (Fig. 1; Tables 1 and 2), a complex Trypticase-yeast extract-containing medium was used (routine growth medium [10]). A chemically defined medium (5) without added nitrogen and reduced with sodium sulfide was used for experiments examining the utilization of nucleosides as nitrogen sources. Glucose, ribose, and deoxyribose were added to complete medium prior to inoculation. Media containing DNA, RNA, or nucleosides as substrates were transferred to an anaerobic glove box (Coy Laboratory Products Inc., Ann Arbor, Mich.), where the substrates were added to boiled, heat-stable medium components and filter sterilized (0.2-μm membrane; Nalgene disposable filterware). Bases were added to media just before sterilization (121°C, 20 min). Carbohydrates were added to media to a final concentration of 0.4% (wt/vol) and nucleosides and bases were added at 0.5% (wt/vol). Media were also supplemented with hemin and 1,4-naphthoquinone when growing *Bacteroides ruminicola* strains and *Succinivibrio dextrinosolvens* 22b, respectively (10). Inocula for these experiments were from overnight glucose-grown cultures. Growth in experimental media was confirmed both by comparison to inoculated media without added energy source and by further transfer of cultures exhibiting growth into experimental media. Growth was monitored spectrophotometrically (Spectronic 70; Bausch & Lomb, Inc., Rochester, N.Y.) over 48 h (3, 6, 12, 24, and 48 h) by determining the optical densities of cultures at 660 nm. Growth measurements were made directly on culture tubes. Because of this, high optical density values are outside the linear relationship with mass. These values, however, were very reproducible and demonstrate the high capacity for growth of these cultures.

DNA (degraded free acid), RNA (type VI from *Torula* yeast), nucleosides, and bases were purchased from Sigma Chemical Co., St. Louis, Mo. All other chemicals were reagent or biological grade. The total carbohydrate, RNA, and DNA contents of yeast extract were estimated by using anthrone (6), orcinol (22), and diphenylamine (2) reagents, respectively. In addition to these analyses, the purity of the DNA and RNA used as growth substrates was confirmed by determining the neutral sugar composition of these materials as described in reference 24.

Preliminary experiments with *S. ruminantium* HD4 showed that complex media (routine growth medium) supported slight but consistent growth in the absence of added energy source. Similar observations have been made by others working with this strain (15). Additional experimentation showed that increasing the concentration of yeast extract resulted in growth increases, while similar responses did not occur with increasing concentrations of Trypticase.

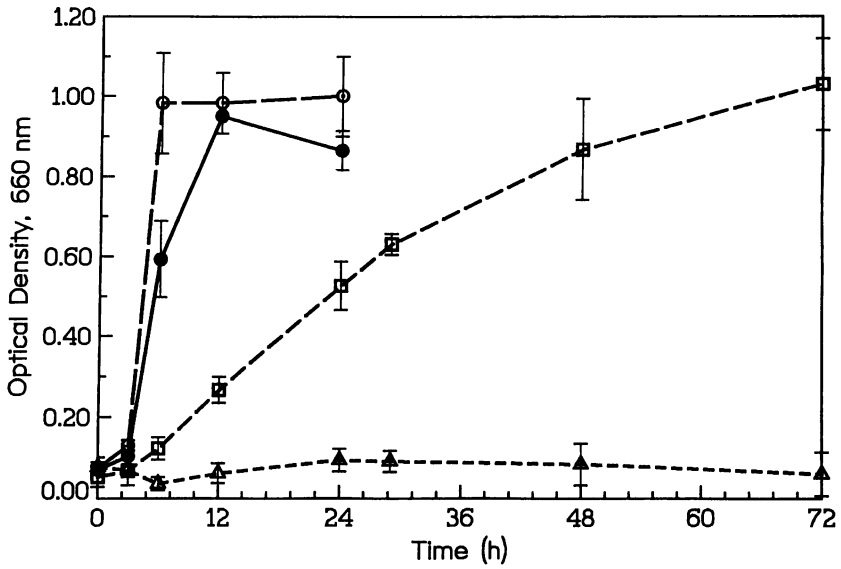

FIG. 1. Growth of *S. ruminantium* HD4 on nucleic acids. Strain HD4 was grown in a medium supplemented with glucose (○), ribose (●), or RNA (□). Data for growth on DNA, deoxyribose, and unsupplemented medium were similar and are indicated by a single symbol (△). Error bars represent standard deviations of triplicate cultures.

Yeast extract contained approximately 9% (wt/wt) carbohydrate, but *S. ruminantium* HD4 was unable to ferment trehalose and glycogen (data not shown; 1, 11), the major storage carbohydrates of Baker's yeast (14, 18). Further analysis of yeast extract showed that it also contained approximately 10% (wt/wt) RNA and 0.1% (wt/wt) DNA. To examine whether *S. ruminantium* HD4 might be able to utilize nucleic acids, this strain was inoculated into a complex medium (routine growth medium) containing DNA or RNA and their component sugars, deoxyribose and ribose. Strain HD4 was able to ferment RNA and ribose, but was unable to grow when provided with DNA or deoxyribose (Fig. 1). Growth on ribose was rapid and compared well with growth on glucose by this strain. Although the growth rate on RNA was much lower than on either glucose or ribose, these cultures did eventually grow to high densities.

A variety of ruminal bacteria, including other strains of *S. ruminantium*, were tested for ability to grow in a complex medium (routine growth medium) containing nucleic acids as energy sources (Table 1). Of the strains studied, one strain of *B. ruminicola* and three *S. ruminantium* strains exhibited the greatest capacities for growth on nucleic acids. All of these strains grew to high densities when provided with RNA for growth. The ability to ferment RNA in the *S. ruminantium* strains appeared to be associated with their ability to use ribose, but *B. ruminicola* D31d grew poorly on ribose even though it could ferment RNA. In addition to these strains, *Streptococcus bovis* JB1 and *Ruminococcus flavefaciens* FD1 could grow on RNA, but this growth was very poor (Table 1).

None of the strains tested were able to effectively utilize DNA, although *R. flavefaciens* FD1 and *Treponema bryantii* did exhibit slight growth on this substrate or on deoxyribose (Table 1). Strains of *Succinivibrio dextrinosolvens*, *Megasphaera elsdenii*, *Lachnospira multiparus*, *R. albus*, *Butyrivibrio fibrisolvens*, and other strains of *B. ruminicola* failed to grow on either RNA or DNA.

The RNA-fermenting strains, *B. ruminicola* D31d and *S. ruminantium* strains HD4, D, and GA192, were able to grow to various degrees when provided with nucleosides (Table 2). However, none of these strains were able to grow on bases alone. Again, *B. ruminicola* D31d exhibited little growth in ribose-containing medium but grew well when provided with nucleosides. No clear preference could be

TABLE 1. Growth of ruminal bacteria on nucleic acids

Species[a]	Strain	Growth with added energy source[b]				
		Glucose	RNA	DNA	Ribose	Deoxyribose
Bacteroides ruminicola	D31d	1.20	1.00	NG[c]	0.05	NG
Streptococcus bovis	JB1	1.00	0.16	NG	NG	NG
Ruminococcus flavefaciens	FD1	1.00	0.16	0.17	0.19	0.14
Treponema bryantii	B_25	0.85	NG	0.21	0.18	0.17
Selenomonas ruminantium	HD4	1.00	1.00	NG	0.95	NG
Selenomonas ruminantium	GA192	0.93	0.93	NG	0.86	NG
Selenomonas ruminantium	D	1.10	0.90	NG	0.66	NG

[a] Other organisms that grew in test medium with glucose, ribose, or deoxyribose but failed to grow on nucleic acids include *B. ruminicola* B_14 and 23, *Succinivibrio dextrinosolvens* 22b, *Megasphaera elsdenii* T81, *Lachnospira multiparus* D32, *Ruminococcus albus* 7, *Butyrivibrio fibrisolvens* D1, CF3, 49, 113, and A38, and *Butyrivibrio*-like strain B385-1.

[b] Growth equals culture optical densities (660 nm; 18 by 150-mm tubes) and are corrected for the small amount of growth supported by the growth medium without added energy source (<0.10). Values are means of triplicate cultures.

[c] NG, No growth.

TABLE 2. Growth of selected ruminal bacteria on bases and nucleosides from RNA

Species	Strain	Growth with given substrate[a]					
		Ribose	Adenosine	Guanosine[b]	Uridine	Cytidine	Bases
B. ruminicola	D31d	0.02	0.88	0.24	0.68	0.07	NG[c]
S. ruminantium	HD4	1.00	0.68	0.57	0.78	0.16	NG
S. ruminantium	GA192	1.10	0.69	0.45	0.42	0.34	NG
S. ruminantium	D	0.76	0.87	0.29	0.71	0.62	NG

[a] Growth equals culture optical densities (see footnote *b*, Table 1).
[b] Guanosine was poorly soluble in media. Nevertheless, a portion was sterile filtered, and the resultant growth on this medium is reported here. A separate portion was not filtered, and growth of all four strains in this turbid medium was confirmed by microscopic examination. All of these organisms exhibited growth in this medium.
[c] NG, No growth.

observed with regard to growth by these strains on either purine- or pyrimidine-containing nucleosides.

To evaluate whether RNA-fermenting bacteria could utilize nucleosides as a sole source of nitrogen and energy, *B. ruminicola* D31d and the three *S. ruminantium* strains were grown in a chemically defined medium containing various nucleosides. Growth in these media was compared with that in glucose and nucleoside media or glucose-plus-ammonia medium. All strains grew well in a medium containing glucose with ammonia, indicating that the defined medium would support growth of these species (Table 3). *B. ruminicola* D31d was unable to utilize nitrogen from nucleosides as shown by its failure to grow in all media when nucleosides were added as sole source of nitrogen. In general, the *S. ruminantium* strains were able to utilize nitrogen from nucleosides since all grew in media containing these compounds when also provided with glucose as an energy source. However, these strains often grew very poorly or not at all when required to extract both nitrogen and energy from nucleosides. Adenosine proved to be the best substrate, supporting good growth of strain GA192 and moderate growth of the other two *S. ruminantium* strains. Strains D and GA192 of *S. ruminantium* were able to use guanosine as a sole source of energy and nitrogen. Uridine and cytidine were poor growth substrates, supporting little if any growth by any of the organisms tested (Table 3). These data suggest that *S. ruminantium* might differ in its capacity for utilization of purine and pyrimidine nucleosides.

TABLE 3. Utilization of nucleosides as a source of nitrogen by selected strains of ruminal bacteria

Nucleoside	Glucose[b]	Growth of given species and strain[a]			
		B. ruminicola D31d	*S. ruminantium* HD4	*S. ruminantium* GA192	*S. ruminantium* D
None[c]	Yes	1.30	0.87	0.76	0.88
Uridine	None	0.00	0.00	0.06	0.00
	Yes	0.00	0.30	0.36	0.16
Cytidine	None	0.00	0.00	0.14	0.04
	Yes	0.00	0.80	0.60	0.40
Adenosine	None	0.00	0.29	0.94	0.20
	Yes	0.00	1.30	1.03	1.20
Guanosine[d]	None	0.00	0.00	0.23	0.31
	Yes	0.00	0.33	0.83	0.30

[a] Growth equals culture optical densities (660 m; 18 by 150-mm tubes). Values are means of triplicate cultures.
[b] Yes indicates that glucose was added to this medium. None indicates that nucleoside served as the sole energy and nitrogen source in the medium.
[c] None here refers to a complete defined medium containing glucose as the energy source and ammonia (10 mM) as the nitrogen source.
[d] Guanosine was poorly soluble in media. Nevertheless, a portion was sterile filtered, and the resultant growth on this medium is reported here. A separate portion was not filtered, and growth in this turbid medium was confirmed by microscopic examination. All of these organisms exhibited growth in this medium.

Based on the results of this study, the occurrence of nucleic acid-fermenting strains among predominant species of ruminal bacteria is low, limited to *B. ruminicola* and *S. ruminantium*. Widespread use of nucleic acids by *B. ruminicola* strains seems somewhat unlikely since other strains tested were unable to grow under these conditions. However, all strains of *S. ruminantium* were able to grow on RNA; thus, it seems likely that this is a characteristic of *S. ruminantium*. Whether or not bacterial strains specialized in nucleic acid fermentation exist in the rumen is unknown, but this seems unlikely since organisms using this survival strategy (i.e., narrowly adapted for use of a particular substrate) usually attack the more abundantly available substrates such as plant polysaccharides and proteins (12).

Although nucleic acids may not always be present at high concentration in the rumen, they do provide those species that can utilize these compounds with an alternate source of energy and nitrogen. Furthermore, the ability of these species and others to utilize nucleic acids as a source of preformed monomers for macromolecular synthesis has not been addressed. This could offer those species able to assimilate these compounds a savings in ATP requirement for their synthesis, resulting in as much as a 14% decrease in ATP for overall cell biosynthesis (8).

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