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Isolation and Characterization of *Selenomonas ruminantium* Strains Capable of 2-Deoxyribose Utilization

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Microbes from ruminal contents of cattle were selectively enriched by using 2-deoxyribose (2DR) as a substrate for growth. Bacterial isolates growing on 2DR were gram-negative, curved, motile rods. The isolates grew on a broad range of substrates, including deoxyribose, glucose, ribose, mannitol, and lactate as well as ribonucleosides and deoxyribonucleosides. The strains also grew on rhamnose (6-deoxymannose) but not DNA. Organic acids produced from growth on hexoses and pentoses included acetate, propionate, lactate, and succinate. The isolates were identified as *Selenomonas ruminantium* subsp. *lactilytica* on the basis of morphology, substrate specificity, and other biochemical characteristics. Several characterized species of ruminal bacteria were also screened for growth on 2DR, with only one strain (*S. ruminantium* PC-18) found able to grow on 2DR. Ethanol was produced by 2DR when strains were grown on ribose or 2DR.

Nucleic acids are a significant substrate for rumen fermentation, given their ubiquitous presence in plant material, microbial biomass, and sloughed ruminal epithelium. In general, nucleic acids are rapidly degraded in the rumen into nucleotides, nucleosides, purine and pyrimidine bases, and the component carbohydrates, ribose and 2-deoxyribose (2DR) (18, 19). However, the microbiology of ruminal nucleic acid utilization and degradation is incomplete. It is known that entodiniomorphid protozoa of the rumen can incorporate nucleotides, nucleosides, and bases into protozoal nucleic acids (8), and several bacterial species have been reported to use these nucleic acid substituents as a source of carbon and nitrogen (9, 15). The metabolism of nucleic acids by ruminal bacteria has recently been investigated (9), and a portion of that work consisted of screening selected species of ruminal bacteria for growth on 2DR. Most strains were unable to grow on this substrate. Two strains with limited growth potential were identified, but most significantly, none of the 19 strains tested achieved high cell densities on 2DR. These results did not support the assumption of McAllan (18) that 2DR would be rapidly metabolized by many species of ruminal bacteria. The objective of the study reported here was to isolate and describe ruminal microbes specifically selected for growth on 2DR.

MATERIALS AND METHODS

Media. Enrichment cultures were established from serially diluted bovine rumen contents by using a 2DR-containing (5 mM) basal anaerobic medium containing, per 100 ml, 5 ml each of mineral solutions 1 and 2 (7), 10 ml of substrate-depleted clarified rumen fluid (10), 0.1 ml of resazurin solution (0.1% [wt/vol]), and 2 ml of cysteine-HCl–sodium sulfide solution (1.25% [wt/vol] each), with a 100% CO_2 atmosphere and 0.4% (wt/vol) (final concentration) Na_2CO_3 equilibrated to a pH of 6.8 (13). Microbes metabolizing 2DR were first isolated after incubation at 39°C from colonies grown in anaerobic roll tubes containing basal anaerobic medium, 5 mM 2DR, and 2% (wt/vol) agar. Secondary isolation of clones was completed by using agar bottle plates (12) that contained the same medium. Cultures were maintained on 2DR-containing (5 mM) modified basal anaerobic medium in which clarified rumen fluid (13), 20% (vol/vol), replaced the substrate-depleted clarified rumen fluid component. Isolates were preserved with glycerol (20% [vol/vol], final concentration; −70°C [28]) after growth on maintenance medium.

Screening medium used for determining 2DR utilization by pure cultures of ruminal bacteria consisted of modified basal anaerobic medium with 5 mM 2DR added. Substrate utilization tests and end product analysis of isolates were conducted in modified basal anaerobic medium with substrates added individually or in selected combinations from filter-sterilized stock solutions stored under nitrogen. Because of their low solubility, stock solutions of nucleosides (100 mM) were warmed before use to approximately 90°C. Stock solutions of adenosine and guanosine were restricted to 40 mM because of lower solubility limits. All nucleoside stock solutions were dispensed (while warm) by syringe to individual culture tubes. At final culture concentrations (4 to 10 mM), all nucleosides except guanosine were soluble in the growth medium.

Ruminal populations of 2DR-utilizing microbes were estimated by inoculating serial dilutions of bovine rumen fluid into modified basal anaerobic medium containing a single substrate (glucose, 2DR, or 2-deoxyglucose [2DG] at a final concentration of 5 mM). Growth was measured in triplicate culture tubes of each dilution by monitoring optical density at 600 nm (OD_{600}). After incubation at 39°C for 7 days, residual deoxy sugar concentrations were determined (2DR- and 2DG-containing tubes only) from culture supernatants by the colorimetric methods of Blecher and Dvorkin (3). Serial dilutions were scored positive when an increase in culture turbidity correlated with the disappearance of the deoxy sugar.

Sources of cultures. The following cultures were obtained from the American Type Culture Collection (Rockville, Md.): *Lactobacillus ruminis* RF1, *L. vitulinus* CL1 and RL2, *Megasphaera elsdenii* T81 and LC1, *Selenomonas ruminantium* GA192, *Eubacterium limosum* 8486, and *Veillonella alcalescens* ATCC 27215. *S. ruminantium* H18 and *Streptococcus bovis* JB1 were obtained from M. Cotta (U.S. Department of Agriculture [USDA], Peoria, Ill. [24, 27]), and *Clostridium longisporum* B6405 was obtained from V. Varel

(USDA, Clay Center, Nebr. [31]). *Treponema bryantii* RUS-1 was obtained from T. Stanton (USDA, Ames, Iowa [26]). All other cultures were isolates reported by M. P. Bryant (5) and were available in the National Animal Disease Center's anaerobe collection.

Analysis. Growth was measured as OD_{600} in 18- by 150-mm culture tubes in a Spectronic 70 spectrophotometer (Milton Roy Co., Rochester, N.Y.).

Morphology of bacterial isolates was determined by phase-contrast microscopy and electron microscopy. Log-phase cultures were harvested, washed in distilled water, and submitted to the National Animal Disease Center microscopy laboratory for preparation and examination. Phosphotungstic acid (0.5%) was used as a negative stain, and stained preparations were examined with a Philips 410 transmission electron microscope.

Additional physiological characteristics were determined by using commercial test kits (API An-Ident and API 20A; Analytab Products, Plainview, N.Y.).

After growth, cell-free supernatants were analyzed for fermentation end products. Organic acids and alcohols were determined by gas-liquid chromatography as previously described (1).

Headspace gas was also analyzed after growth for hydrogen and carbon dioxide by previously described methods using gas chromatography (1).

High-performance liquid chromatography (HPLC) of monosaccharides was performed by using a Beckman System Gold fitted with a Waters model 410 refractive index detector and an Interaction CHO-620 carbohydrate column (Interaction Chemicals, Mountain View, Calif.) heated to 90°C. HPLC-grade water was used as the eluent at a flow rate of 0.5 ml/min. When required, samples were desalted by treatment with ion-exchange resins to avoid damage to the HPLC column according to the methods of Russell (23). Maltose was used as an internal standard.

The moles percent G+C of phenol-extracted cellular DNA (17) was determined by thermal denaturation, with DNA from *Escherichia coli* used as a reference (16).

Hydrolysis of DNA was determined by agarose gel electrophoresis. Cultures were grown in modified basal anaerobic medium containing salmon testis DNA (Sigma) and in some cases ribose or 2DR. Stock solutions of DNA were prepared in sterile, anaerobic (with N_2 as the headspace gas) water but were not filter sterilized. Uninoculated tubes of medium containing DNA did not indicate growth of contaminants during the incubation period of 7 days. Cell-free culture supernatants were then prepared for electrophoresis according to the procedures of Maniatis et al. (17). Agarose gels were inspected for changes in migration patterns which would indicate DNA hydrolysis and greater mobility of fragmented DNA.

RESULTS AND DISCUSSION

A diverse range of previously characterized ruminal bacteria were screened for growth on 2DR. Only one strain (*S. ruminantium* subsp. *lactilytica* PC-18 [4]) was able to grow on 2DR as the sole carbon substrate. Other strains of *S. ruminantium* (HD1, HD4, H18, and GA192) did not grow on 2DR. Other bacteria which failed to grow on 2DR included *Ruminococcus flavefaciens* C94, *Eubacterium ruminantium* B_4, *M. elsdenii* B159, LC1, and T81, *Ruminobacter amylophilus* 70, *Prevotella ruminicola* 23, *Butyrivibrio fibrisolvens* D1, *Succinivibrio dextrinosolvens* 22B, *Lachnospira multiparus* D32, *Propionibacterium acnes* 8586A, *Succinimonas amylolytica* $B_2$4, *T. bryantii* RUS-1, *Fibrobacter succinogenes* S85, *Ruminococcus albus* 7, *L. ruminis* RF1, *L. vitulinus* RL2 and CL1, *E. limosum* 8486, *Streptococcus bovis* JB1, *V. alcalescens* ATCC 27215, and *C. longisporum* B6405.

TABLE 1. Taxonomic characteristics of 2DR-utilizing isolates

Characteristic	Detection in[a]:				
	S. ruminantium[b]	SC-1	PC-18	JM-3	JM-4
Catalase	−	−	−	−	−
Gelatin	v	+	−	−	−
Motility	+	+	+	+	+
Indole	−	−	−	+	+
Hemolysin[c]	v	+	ND	+	+
Heat resistant[d]	−	−	−	−	−
Gas[e]	+	+	+	+	+
Sulfide	v	−	+	−	−
Acetoin	v	−	+	+	+
Sedimentation	+	+	+	+	+

[a] +, detected; −, not detected; v, variable dependent upon strain; ND, not determined.
[b] Data compiled from references 4, 6, and 20.
[c] Alpha hemolysis observed on anaerobic blood agar plates.
[d] 80°C, 5 min.
[e] As determined from growth of new isolates on glucose and ribose.

Cotta (9) tested 19 strains of ruminal bacteria for growth on 2DR. All of the three strains of *S. ruminantium* tested (HD4, GA192, and D) failed to grow. Two other strains of bacteria (*R. flavefaciens* FD1 and *T. bryantii* $B_2$5) that could grow on 2DR were found, but their growth was very limited. All other strains failed to grow on this substrate (9).

Low population densities of 2DR-degrading microbes (less than 10^6/ml) were found in rumen contents collected from cattle at the National Animal Disease Center. 2-Deoxyglucose (2DG) was not metabolized by mixed ruminal microbes, as all dilutions failed to grow and 2DG levels in the culture medium did not decrease.

The 2DR-degrading strains (SC-1, JM-3, and JM-4) isolated from enrichment cultures of bovine rumen contents were anaerobic, gram-negative (by stain), curved rods with tapered ends. Motility was observed in all strains, and cells examined by electron microscopy exhibited lateral flagellation typical of the *Selenomonas* sp. (6). The moles percent G+C for the new isolates was 52.3 ± 1.9. This value was comparable to that determined for strain PC-18 (53.9%) and similar to previously reported values (54%) for this species (6, 20). Other taxonomic characteristics of the isolates were similar to those of *S. ruminantium* (Table 1), although some variation was observed (6, 21, 29).

A wide range of substrates supported growth of individual strains. Substrates utilized by all four strains included arabinose, cellobiose, fructose, galactose, glucose, glycerol, lactate, lactose, mannitol, ribose, sucrose, xylose, esculin, maltose, and salicin. The strains varied in use of other substrates (Table 2). The only deoxy sugars that supported growth were 2DR, rhamnose (6-deoxymannose), and to a limited extent fucose (6-deoxygalactose) and 2-deoxygalactose (Table 3). *P. ruminicola* $B_1$4 has been reported to degrade rhamnose, with 1,2-propanediol being produced during rhamnose fermentation (30). The activity of *P. ruminicola* was limited, however, since the deoxy sugar fucose did not support growth. Wallace (32) reported limited degradation (<6% of initial concentration) of the deoxy sugars fucose and rhamnose when these substrates were incubated

TABLE 2. Profile of substrate utilization for strains of *S. ruminantium* capable of growth on 2DR

Substrate[a]	Growth[b]				
	S. ruminantium[c]	SC-1	PC-18	JM-3	JM-4
Adonitol	−	−	ND	+	+
Amino acids	−	ND	−	+	+
Cellulose	−	−	−	−	−
Dulcitol	+	−	+	+	+
Erythritol	v	−	ND	+	+
Inositol	−	+	ND	+	+
Inulin	v	+	−	+	+
Mannose	+	+	ND	+	+
Melezitose	−	−	ND	+	+
Melibiose	+	+	ND	+	+
Pyruvate	v	ND	ND	+	+
Raffinose	+	+	ND	+	+
Rhamnose	v	+	ND	+	+
Sorbitol	−	−	ND	+	+
Starch	+	+	−	+	+
Trehalose	−	+	−	+	+

[a] Final concentration of each substrate was 1% (wt/vol).
[b] +, growth; −, no growth; v, variable growth; ND, not determined.
[c] Data compiled from references 4, 6, and 20.

TABLE 4. Organic acids produced by *S. ruminantium*

Substrate[a]	Strain	Concn (μmol/ml)[b]			
		Acetic acid	Propionic acid	Lactic acid	Succinic acid
Ribose					
	JM-3	0.9	1.4	3.2	0
	JM-4	1.5	1.7	2.2	0
	PC-18	0.4	0.6	6.0	0
2DR					
	JM-3	2.0	2.0	0	0
	JM-4	0.8	0.7	0	0
	PC-18	0.8	0.4	0	0.6
Glucose					
	JM-3	1.4	1.5	7.0	0
	JM-4	0.2	0.2	7.2	0
	PC-18	1.0	0.2	5.5	0
Fructose					
	JM-3	2.2	2.2	24.0	0
	JM-4	1.1	0.9	10.8	0
	PC-18	0.6	0.2	9.2	0

[a] Initial concentrations for substrates: glucose, 2DR, and ribose, 15 mM; fructose, 1% (wt/vol).
[b] In cell-free supernatant after 24 h of growth. Growth was >1.0 (OD_{600}) for all strains and substrates except PC-18 grown on 2DR, in which case the OD_{600} was 0.4.

with ruminal inocula for 1 h. Deoxyribose was not included in either of these studies.

Growth was observed on both deoxyribonucleosides and ribonucleosides (Table 3). These observations agree with previous results which indicated that strains of *S. ruminantium* degraded ribonucleosides (adenosine, guanosine, uridine, and cytidine) (9). Superior growth was observed on nucleosides containing the purine bases guanine and adenine. The 2DR-utilizing strains (JM-3, JM-4, and PC-18) were unable to grow on the purine or pyrimidine bases when these were provided as the sole substrate. However, other strains of *S. ruminantium* were reported to use free bases as well as nucleosides as a source of nitrogen when glucose was added to a nitrogen-free medium (9, 15).

TABLE 3. Utilization of nucleosides, deoxynucleosides, and deoxy sugars by strains of *Selenomonas ruminantium*

Substrate	Growth[a]			
	SC-1	PC-18	JM-3	JM-4
Deoxyribonucleosides				
Deoxyguanosine (4 mM)	>1.0	>1.0	>1.0	>1.0
Deoxyadenosine (4 mM)	0.60	0.34	>1.0	>1.0
Deoxyuridine (10 mM)	−	−	0.23	0.32
Deoxycytidine (10 mM)	−	−	0.56	0.48
Thymidine (10 mM)	0.40	0.52	0.54	0.60
Ribonucleosides				
Guanosine[b] (4 mM)	+	−	+	+
Adenosine (4 mM)	0.90	0.34	0.70	0.68
Uridine (4 mM)	0.43	0.29	0.42	0.52
Cytidine (4 mM)	0.36	ND	0.54	0.58
Deoxy sugars				
2DR (1% [wt/vol])	0.80	0.50	>1.0	>1.0
3DR (5 mM)	−	−	−	−
Fucose (1% [wt/vol])	−	−	0.19	0.24
Rhamnose (1% [wt/vol])	0.81	ND	0.80	0.90
2-Deoxygalactose (1% [wt/vol])	−	0.12	0.13	0.15
2DG (1% [wt/vol])	−	−	−	−
6DG (5 mM)	−	−	−	−

[a] OD_{600} after 24 h of growth (18- by 150-mm culture tubes), corrected for residual growth on basal medium. +, growth; −, no growth (<0.1 OD_{600}); ND, not determined.
[b] Guanosine precipitated in the medium. Growth was determined by microscopic examination.

Our strains of *S. ruminantium* did not grow on intact DNA (0.75 mg/ml), and no evidence of hydrolysis was apparent when DNA incubated with growing cultures was examined by agarose gel electrophoresis. In contrast, rapid ruminal DNA hydrolysis has previously been observed, and numerous other species are capable of such degradation (11).

The newly isolated 2DR-using strains exhibited similar responses in the ability to grow on several substrates. Specific growth rates (per hour) and maximal growth (OD_{600}) of strain JM-4 in cultures containing equimolar (4 mM) substrate concentrations were as follows: with glucose, 0.53 and 1.2; with ribose, 0.75 and 0.9; and with 2DR, 0.35 and 0.6, respectively. Growth yields for strain JM-4 grown on glucose, ribose, and 2DR were 60, 41, and 23 g/mol (dry weight), respectively.

Growth of strain JM-4 on 2DR (4 mM) plus ribose (4 mM) or 2DR (4 mM) plus glucose (2 mM) did not exhibit a diauxic pattern, and 2DR utilization was not delayed by the presence of the other substrates. Rates of 2DR utilization and maximum culture OD_{600} for this isolate when grown on 2DR only, 2DR plus ribose, and 2DR plus glucose were 0.52, 0.63, and 0.69 μmol/ml/h and 0.5, 0.9, and 1.0, respectively.

Organic acids produced by the 2DR-using strains of *S. ruminantium* were typical of the species (6, 20) and included acetate, propionate, lactate, and traces of succinate (Table 4). Carbon dioxide was also produced, but H_2 was not detected in samples of fermentation gases. Ethanol was also detected in cultures grown on either 2DR or ribose (Table 5). However, ethanol was not detected when cultures were grown on glucose, an observation previously reported for strain PC-18 (4).

Bacterial production of ethanol from pentoses and deoxypentoses has been previously described, and the action of 2-deoxyribose-5-phosphate aldolase (EC 4.1.2.4) has been implicated as key to this conversion (2, 14, 22, 25). For 2DR, the activity of this aldolase results in hydrolysis of 2DR-5-

TABLE 5. Concentration of ethanol produced by 2DR-degrading strains of *S. ruminantium* grown on 2DR or ribose

Strain	Substrate (4 mM)	Incubation time (h)	Ethanol concn (μmol/ml)[a]
JM-4	2DR	0	0
		6	0
		12	0.5
		24	0.8
		48	1.2
PC-18	2DR	0	0
		6	0
		12	0
		24	0.6
		48	0.6
JM-3	Ribose	0	0
		6	0
		12	0.5
		24	1.7
		48	3.8

[a] In cell-free supernatant of modified basal anaerobic medium with substrate added as indicated.

phosphate into glyceraldehyde-3-phosphate and acetaldehyde. The deoxy carbon is thus preserved in acetaldehyde and after reduction in ethanol. It is not known whether this enzyme is present in *Selenomonas* spp., but the energy lost from the disposal of this two-carbon metabolite could be responsible for the lowered yields observed from growth on 2DR.

Growth of *Selenomonas* strains on deoxyribonucleosides and 2DR demonstrates the presence of a major metabolic route for nucleic acid metabolism. Such enzymes are ubiquitous for the catabolism and recycling of endogenous nucleic acids, and such activity is widely reported in the literature (11, 22, 25). However, in certain microbial species, such activity may result in the metabolism of exogenous nucleic acids (9, 33). In these cases, nucleic acid components can serve as a source of energy for growth and as a major source of carbon and nitrogen for biosynthesis.

In the rumen, nucleic acid catabolism reflects the general versatility that exists with regard to substrate utilization, and 2DR use is but one component of this metabolic process. The presence of ruminal microbes which can utilize 2DR is not surprising, given the quantities of nucleic acids that are present in the diet (18). However, the relatively low population density of 2DR-using microbes that was observed may be a reflection of the low concentration of free 2DR that is available in the rumen. Nucleic acids are rapidly degraded in the rumen, and components such as nucleotides and nucleosides can transiently appear (19). Previous research has demonstrated that nucleic acid metabolism and incorporation can occur at the oligonucleotide, nucleotide, and nucleoside levels, thus placing limits on the quantity of free 2DR (8). Deoxyribose-using *Selenomonas* strains with their broad substrate versatility certainly contribute to ruminal metabolism and to the nutritional well-being of the host animal; however, their numbers may be restricted by substrate availability. If substrate concentrations were to increase, a concomitant increase in their numbers and activity would be expected.

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