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Glucose-1-Phosphate as a Selective Substrate for Enumeration of *Bacteroides* Species in the Rumen[1]

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When glucose-1-phosphate was used as the only added energy source in a selective roll tube medium, colony counts for rumen contents ranged from 17.8 to 84.8% of the total culturable count. Percentages were highest in rumen contents from sheep fed high-concentrate rations. From a total of 73 cultures isolated from glucose-1-phosphate roll rubes, only 15.1% were presumptively identified as *Bacteroides* species. Strains presumptively identified as *Butyrivibrio, Selenomonas, Treponema, Streptococcus bovis,* and *Lachnospira* also fermented glucose-1-phosphate. Thus, glucose-1-phosphate would not be useful as a selective substrate for isolation or enumeration of *Bacteroides* species from the rumen.

Wilkins and Walker (7) tested 1,504 strains of anaerobic bacteria, from 12 different genera, for their ability to produce acid from glucose-1-phosphate (G1P). Out of 544 strains that were positive, 535 were fermentative strains of *Bacteroides* and nine were clostridial strains. Only three fermentative *Bacteroides* strains were negative. Since a basal selective roll tube medium for the enumeration of anaerobic rumen bacteria fermenting specific substrates had recently been developed in this laboratory (4), it suggested the possibility that using G1P as a substrate might allow direct determination of the numbers of *Bacteroides* species in rumen contents. The results of this study constitute the present report.

The basal medium, its preparation, and anaerobic roll tube techniques have been described previously (4, 5). Total colony count was determined in the basal medium containing 0.05% each of glucose, cellobiose, xylose, and soluble starch (4). G1P (dipotassium salt, Nutritional Biochemicals Corp.) was added to the basal medium before sterilization at a 0.2% (wt/vol) concentration. Glucose could not be detected in the G1P with the glucose oxidase assay (Glucostat, Worthington Biochemicals Corp.). Autoclaving in water for 20 min at 121°C did not hydrolyze G1P; however, analysis of the G1P-containing roll tube medium after autoclaving gave a very slight positive color reaction for glucose, which, if all free glucose, would have given a maximum concentration of only 0.0075% in the medium. This concentration would equal 3.7% hydrolysis of G1P.

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Samples of rumen contents were obtained from fistulated sheep, each fed daily 800 g of one of the rations listed in Table 1 plus 20 g of a mineral-vitamin mix and 4.4 g of feed-grade urea. The animals were fed once daily at 9:00 a.m., and samples were collected just before feeding.

The total culturable colony count, G1P colony count, and G1P colony count as a percentage of total count for rumen contents from sheep fed four different rations are presented in Table 1. The percentage of colonies growing in G1P medium increased markedly as the amount of concentrate in the ration increased, i.e., from 17.8% with mixed hay to 84.8% for the 90% corn ration.

TABLE 1. *Comparison of total culturable colony counts and colony counts on G1P medium in rumen contents of sheep fed various rations*[a]

Rations	Total colony count[b]	G1P colony count[b]	G1P count as % of total
MH[c]	52.5	9.3	17.8
Alfalfa	103.8	34.2	33.0
60% corn-40% MH	74.8	49.8	66.6
90% corn-10% MH	84.1	71.3	84.8

[a] Substrates added to the basal selective medium for total counts were 0.05% each glucose, cellobiose, xylose, and soluble starch (4). G1P medium contained 0.2% G1P.

[b] Colony counts were for 10^{-8} g of rumen contents.

[c] MH, Mixed hay.

Colonies picked from roll tubes of G1P medium, inoculated with dilutions of rumen contents from sheep fed either mixed hay or 90% corn, were stabbed into 40% rumen fluid-glu-

cose-cellobiose-agar (RGCA) slants (2). However, the fact that one rumen *Bacteroides* species, *B. amylophilus*, can ferment only maltose or starch had been overlooked (6). Therefore, cultures picked from G1P roll tubes inoculated with dilutions of rumen contents from sheep fed alfalfa or 60% corn ration were stabbed into RGCA plus starch (RGCSA) slants (5). In addition to examination of wet mounts by phase-contrast microscopy, Gram stain, anaerobiosis, motility, and H_2S production were determined on each isolate (3). Wilkins and Walker (7) established a positive reaction for G1P fermentation as a decrease of 0.8 to 0.9 pH units after 72 h of incubation. However, it appeared possible that some organisms might be able to partially or slowly ferment G1P, at least enough to produce visible colonies in roll tubes after 7 days of incubation. Using poorly buffered 0.5% G1P broth containing 40% rumen fluid as a test medium (2), fermentation of G1P by the isolated strains was estimated as follows: (i) a decrease in pH after 7 days of incubation; (ii) increase with time in optical density (OD) at 600 nm; and (iii) disappearance of G1P, based on loss of total hexose as measured with the

TABLE 2. *Establishment of criteria for the fermentation of G1P by isolated strains*[a]

G1P disappearance (%)	Increase in OD at 600 nm	Decrease in pH (units)
>75% = 42[b]	>0.6 = 5	>0.8 = 9
<75% > 25% = 10	<0.6 > 0.3 = 16	<0.8 > 0.5 = 36
<25% > 15% = 1	<0.3 > 0.15 = 28	<0.5 > 0.15 = 8
≤15% = 7	≤0.15 = 11	≤0.15 = 7

[a] Based on data from 60 strains. The seven strains for which less than 15% G1P disappeared were common to the lowest range in the other two criteria.
[b] Number of strains.

TABLE 3. *Morphological grouping of strains isolated from G1P medium, their relative distribution in sheep fed different rations, and their ability to ferment G1P*

Morphology[a]	Type	MH[b]		Alfalfa		60% corn-40% MH		90% corn-10% MH		All rations		
		No.	G1P+[c]	No.	G1P+	No.	G1P+	No.	G1P+	No.	G1P+	Percent G1P+
Gram negative												
Motile												
Curved rods												
"Butyrivibrio"[d]	I	15	15	5	4	5	3	7	7	32	29	90.6
"Selenomonas"	II	0	0	0	0	2	2	3	3	5	5	100.0
Straight rods	III	4	4	3	3	0	0	0	0	7	7	100.0
"Treponema"	IV	0	0	0	0	2	2	0	0	2	2	100.0
Nonmotile rods												
"Bacteroides"	V	3	3	1	1	5[e]	5	2	1	11	10	90.9
Other (large)	VI	0	0	0	0	4	1	0	0	4	1	25.0
Gram positive												
Coccus												
"Streptococcus bovis"	VII	0	0	0	0	1	1	5	ND[f]	6	1/1[g]	
Gram positive (weak)												
Coccus	VIII	0	0	0	0	0	0	2	0	2	0	0
Motile rods												
In chains	IX	0	0	0	0	3	2	0	0	3	2	66.7
Pointed ends	X	0	0	0	0	0	0	1	0	1	0	0
Total strains		22	22	9	8	22	16	20	11/15[g]	73	57/68[g]	83.8
Percent of strains utilizing G1P		100		88.9		72.7		73.3				

[a] All strains were obligate anaerobes, except for one *"Bacteroides"* isolate (footnote *e*). The five *"S. bovis"* strains listed under 90% corn-10% MH were not tested.
[b] MH, Mixed hay.
[c] Number of strains which can ferment G1P.
[d] Genera inside quotation marks signify presumptive identification based on criteria listed in text.
[e] One strain was classified as a facultative anaerobe.
[f] G1P fermentation was not determined.
[g] G1P-fermenting strains over number of strains tested.

orcinol reaction (1). All three criteria were measured for each individual fermentation tube.

A total of 60 isolates were tested by all three criteria, and the results are summarized in Table 2. The data for each criterion were grouped together in ranges, in order to compare the magnitude of response with distribution of the strains. The seven strains for which less than 15% G1P disappeared were also included in the ≤0.15 increase in OD and ≤0.15 pH unit decrease groups. Thus, any strain causing less than 15% G1P disappearance and having a pH decrease of less than 0.15 pH units was considered negative for G1P fermentation. Using an increase in OD greater than 0.15 to indicate fermentation of G1P does not appear to be completely satisfactory. The distribution of strains in Table 2 suggests that G1P disappearance is probably the best single estimate of G1P fermentation, since 52 of the 60 strains were in the >25% disappearance range. Eight additional isolates, not previously studied, were tested only for disappearance of G1P; four caused ≤15% G1P disappearance, and the other four showed 67.7, 28.4, 71.3, and 63.2% disappearance of G1P, respectively. Based on these data, 11 of the 68 strains tested were listed as negative for G1P fermentation.

The isolates were classified into 10 morphological types, and their distribution and ability to ferment G1P are shown in Table 3. Only 11 strains, or 15.1% of the total number of isolates, were presumptively classified as *Bacteroides*, and all but one strain fermented G1P. By far the largest proportion of isolates, 46, were anaerobic gram-negative motile rods presumptively classified as "*Butyrivibrio*" type, "*Selenomonas*" type, "*Treponema*" type, and "others." Of these, 93.4% fermented G1P. The diversity of morphological types was higher from the sheep fed concentrate-type rations; however,

TABLE 4. *Comparison of G1P fermentation between presumptively identified strains isolated from G1P roll tubes and characterized strains of rumen bacteria*

Strains	Type[a]	Increase in OD at 600 nm	Decrease in pH (units)	G1P disappearance (%)
Presumptively identified				
"Butyrivibrio"				
K4	I	0.21 (48)[b]	0.89	81.0
K41	I	0.79 (16)	0.43	80.2
K47	I	0.09 (17)	0.17	24.5
K60	I	0.06 (17)	0.07	0
K89	I	0.11 (16)	0.16	42.7
"Selenomonas"				
K2	II	0.15 (24)	0.69	83.3
"Treponema"				
K74	IV	0.11 (168)	0.88	35.0
"Bacteroides"				
K24	V	0.40 (168)	0.26	27.6
K39	V	0.89 (17)	0.55	86.4
K77	V	0.34 (144)	0.96	62.2
"Streptococcus bovis"				
K65	VII	0.34 (73)	1.23	86.5
"Lachnospira multiparus"				
K63	IX	0.17 (47)	0.96	84.9
Characterized				
Butyrivibrio fibrisolvens				
E9a		0.07 (16)	0.10	22.3
H10b		0.28 (24)	0.56	81.8
D16f		0.03 (16)	0.19	0
E44a		0.07 (24)	0.13	20.7
Selenomonas ruminantium				
GA192		0.12 (168)	0.29	41.3
L. multiparus				
D15d		0.08 (72)	0.02	0
S. bovis				
E2c		0.05 (24)	0.03	5.7
Bacteroides ruminicola				
H8a		0.94 (24)	0.53	80.2

[a] See Table 3.

[b] The figure in parentheses indicates the hours required to reach maximum optical density.

the percentage fermenting G1P was lower. Five strains isolated from the sheep fed 90% corn were presumptively identified as *S. bovis* and discarded before the need for further tests was realized.

Growth, acid production, and G1P disappearance for several of the presumptively identified isolates from this study and previously characterized strains of rumen bacteria are shown in Table 4. The lack of association between these three parameters both within and among strains is most striking. For example, "*Butyrivibrio*" strains K4 and K41 showed almost equal disappearance of G1P, yet the magnitude of increase in OD and decrease in pH were reversed. "*Treponema*" strain K74 caused a decrease in pH equal to that of strain K4, yet only 35% G1P disappeared and the increase in OD was minimal. These data suggest that use of either increase in OD or decrease in pH as a single criterion for measuring G1P fermentation could be quite misleading. Disappearance of G1P from the culture medium appears to be a valid estimate of fermentation. For all of the cultures examined, *B. fibrisolvens* D16f was the only strain that caused less than 15% disappearance of G1P and exceeded either of the lower limits of ≤ 0.15 increase in OD or ≤ 0.15 decrease in pH proposed earlier.

Based on the data presented, G1P cannot be considered a satisfactory selective substrate in roll tubes for the enumeration of *Bacteroides* species in rumen contents. Even the fermentation of G1P as a screening procedure for distinguishing *Bacteroides* sp. among isolated strains does not appear feasible. Most of the present isolates that fermented G1P were from genera not tested by Wilkins and Walker (7), but are common to the flora of the cecum, colon, rumen, and feces.

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