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Urease Assay and Urease-Producing Species of Anaerobes in the Bovine Rumen and Human Feces

M. A. WOZNY,[1] M. P. BRYANT,* L. V. HOLDEMAN, AND W. E. C. MOORE

Departments of Dairy Science and Microbiology, University of Illinois, Urbana, Illinois 61801, and Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24060*

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A growth medium and test were developed for rapid detection of urease in fermentative anaerobic bacteria. Using nonselective rumen fluid roll-tube agar medium and the new test, it was confirmed that *Peptostreptococcus productus* is often the most numerous urease-forming species in human feces. Also, some fecal strains of *Ruminococcus albus, Clostridium innocuum,* and *Clostridium beijerinckii* produced urease. Single strains of *Fusobacterium prausnitzii, Coprococcus catus,* and *Streptococcus mitis* that were strongly ureolytic on isolation later lost this ability. Urease activity was also detected in many strains of nonselectively isolated rumen species. They include *Succinivibrio dextrinosolvens, Treponema* sp., *Ruminococcus bromii* (not previously known to be present in the rumen), *Butyrivibrio* sp., *Bifidobacterium* sp., *Bacteroides ruminicola,* and *P. productus*. Most *P. productus* strains contain urease; however, the uniformity of this feature in the other species noted above is not known. The urease in many of these species was not detected if the growth medium contained 0.2% or more (each) yeast extract and Trypticase.

Production of ammonia via hydrolysis of urea by bacteria in the rumen and large bowel is important in the nitrogen metabolism of animals (29), but as yet there is inadequate documentation concerning the most numerous species of urease-forming bacteria functional in these habitats (see 19, 28, and references therein). Although a very large proportion of the bacteria in the rumen and large bowel are strict anaerobes, few strictly anaerobic urease-forming species have been documented. The work of Cook (12) suggests that, at least under some conditions in the sheep rumen, a less numerous facultative anaerobe, *Streptococcus faecium,* may be functional because of the relatively high specific activity of its urease even when grown in environments relatively high in availabile nitrogen. Available nitrogen represses urease formation in many bacteria (21, 22).

In recent studies with a selective isolation technique involving chemically defined anaerobic roll-tube medium containing a small amount of urea as sole nitrogen source, relatively large numbers of the urease-forming anaerobes *Selenomonas ruminantium* and *Peptostreptococcus productus* were isolated from the bovine rumen and human feces (19, 28). These species were not previously known to include urease-forming strains, probably because the urease was strongly repressed in media containing the high nitrogen levels used in assay procedures with anaerobes (19, 28). Also, many strains of *S. ruminantium* isolated on nonselective medium do not form urease (19). These studies suggested that urease-forming strains of other anaerobic species may not have been detected either because of their inability to develop large colonies in the chemically defined medium used in primary isolation (19, 28) or because of unsatisfactory assay procedures.

In this paper, we describe a rapid qualitative procedure to detect urease and document its occurrence in many strains of predominant species of strict anaerobes nonselectively isolated from the bovine rumen and human feces.

MATERIALS AND METHODS

The anaerobic techniques for culture, enumeration, and isolation of bacteria were as previously described (2, 11), with exceptions as indicated below. In the rumen fluid-glucose-cellobiose-starch agar roll-tube medium (medium 98-5) used for nonselective enumeration and isolation, the reducing solution was changed from 0.025% each of cysteine-hydrochloride-H_2O and $Na_2S \cdot 9H_2O$ added to individual tubes, as used with rumen samples, to 0.05% of cysteine-hydrochloride-H_2O added to the medium before tubing when culturing fecal samples (14). Rumen fluid carbohydrate maintenance medium slants were as previously described (11), except that

[1] Present address: School of Veterinary Medicine, Auburn, AL 36830.

only 1% agar and 0.1% each of glucose, cellobiose, and starch were used.

The composition of the medium for urease detection is shown in Table 1. In its preparation, the pH was adjusted to 6.5 with 2.5 N NaOH after addition of most ingredients. Dithiothreitol was then added, and the medium was brought to volume minus ingredients added after autoclaving. It was then boiled, stoppered, autoclaved, and cooled under anaerobic conditions. Sterile carbonate, filter-sterilized urea, and sulfide solutions were then added, and the medium was aseptically tubed under CO_2 in 3.0-ml amounts in rubber-stoppered tubes (13 by 100 mm) as previously described (2).

Samples of rumen contents for isolation of bacteria were obtained (2 to 5 h after feeding) from two Holstein cows with fistulated rumens, on diets of about 6.8 kg of alfalfa hay and 3.6 kg of grain mixture (15% crude protein). Other rumen samples were collected by stomach tube 2 to 7 h after feeding cows (dry weight) about 1.9 kg of alfalfa hay, 5.0 to 8.6 kg of corn silage, and 9 to 11 kg of one of three grain mixtures per day. The grain mixture of ration 65 included 95% corn, 1.5% dicalcium phosphate, 2% limestone, 1.2% trace mineralized salt, 0.15% Na_2SO_4, vitamin A and D supplement, and no supplementary nitrogen. Grain mixtures for the other two rations were similar, except that of ration 66 included 1.5% urea and that of ration 67 included 10.75% soybean meal (49% crude protein). The total crude protein of the three rations fed was 8.9, 10.8, and 11.1%. The samples were processed for culture as previously described (11), except that strained fluid samples (ventral rumen) were used and they were not blended. Two fecal samples from each of two 24-year-old humans (one male and one female) on unrestricted diets were collected, processed, and cultured as previously described (14).

TABLE 1. *Composition of the culture medium for urease detection*

Component	%
Glucose	0.1
Maltose	0.1
Cellobiose	0.1
Sodium lactate	0.1
Trypticase (BBL)	0.05
Yeast extract (Difco)	0.05
Mineral solution 3 (28)	5.0
B-vitamin solution (28)	0.5
Hemin solution[a]	0.5
Menadione solution[b]	0.1
Volatile fatty acid solution (28)	0.45
Ferrous sulfate solution[c]	0.2
Resazurin solution (0.1%)	0.2
Dithiothreitol solution (1%)	1.0
Sulfide solution (28)	1.0
Urea solution (1.5 M urea-N)	3.3
Sodium carbonate solution (8%)	5.0
CO_2 gas phase	
Distilled water to volume	

[a] 4 mg of hemin in 80 ml of distilled water plus 20 ml of fresh 10% NaOH.

[b] To an 18- by 150-mm, sterile rubber-stoppered tube, 0.02 g of menadione and 10 ml of absolute ethanol were added, aseptically equilibrated with 100% CO_2, and stored in the dark at 4°C.

[c] To 100 ml of distilled water, 0.218 g of $FeSO_4 \cdot 7H_2O$ plus 3 drops of HCl were added. Storage was at room temperature.

Urease-forming strains were detected as follows. Isolated colonies were randomly picked from roll tubes after 3 or 4 days of incubation at 37°C and stab-inoculated into the maintenance medium slants (11). When good growth was observed (usually after 1 day of incubation), a 0.5-ml volume of control medium (the urease detection medium modified by deletion of urea) was anaerobically added and mixed with the growth, and 0.05 ml was then used to inoculate one tube of the control medium and duplicate tubes of the urease detection medium. These were incubated for 72 h along with uninoculated controls. Media inoculated with urease-forming *S. ruminantium* strain D (19) were included in each test set as a positive control. Bacterial growth was estimated by optical density determinations every 18 to 24 h at 600 nm with a Bausch and Lomb Spectronic 20 (fecal strains) or Spectronic 70 (rumen strains). Ammonia production from urea was then determined using a qualitative spot test. About 0.5 ml of culture was placed in a porcelain spot dish with 2 drops of 2 N NaOH; after mixing, 3 drops of Nessler reagent (27) was added. Ammonia production was recorded on a scale of 0 to 3+, depending on the amount of color and precipitate that developed as compared to the control without urea. Uninoculated media never contained detectable amounts of ammonia, and small amounts of ammonia were detected in the inoculated control medium (minus urea) in only a few instances.

Urease-producing cultures were grouped on the basis of morphology as seen in Gram stains and in wet mounts of young cultures viewed with the phase-contrast microscope. Cultures representative of the groups were more extensively characterized using methods previously described (18).

RESULTS AND DISCUSSION

Urease detection medium. The medium (Table 1) was developed on the basis of preliminary experiments with strain D of *S. ruminantium* and strain 1-1 of *P. productus* (M. A. Wozny, M.S. thesis, University of Illinois, Urbana, 1975) and on the basis of known nutritional requirements of many anaerobic bacteria (see 4 and references therein). The detection of ammonia with the Nessler reagent was more reliable than that by pH indicator (28). The activity of the urease of the two strains (moles of ammonia produced per minute per gram of protein; see Wozny, M.S. thesis, for methods) was depressed two to sixfold by the inclusion of 50 mM urea as compared to inclusion of none, but detection of ammonia was best with 50 mM or more. Maximum growth occurred with 3 to 6 mM urea, whereas 300 mM urea, which is less

than the amount used in many assay systems, inhibited growth of strain 1-1. Ammonia production was not detected with strain D if 0.2% each of yeast extract and Trypticase were added to the medium; only a little production was seen with 0.1% each, but at 0 or 0.05% each of yeast extract and Trypticase, ammonia production was not inhibited.

Concerning nutrients required for growth of anaerobes (4), Trypticase (0.05%) was added to provide peptides and amino acids that might be required by some species. Yeast extract (0.05%) was added to provide tetrahydrofolate and other materials required by some anaerobes but not included in the medium as defined ingredients. The volatile acids, heme, menadione, B-vitamins, and carbonate are known requirements of many anaerobes, as are the three different sugars and lactate. Anaerobes such as methanogens or sulfate reducers, which require high levels of H_2 or sulfate, would obviously not grow well in this medium.

Numbers of urease-forming bacteria. The total viable counts of bacteria and the proportion of the cultures picked that produced urease are shown in Table 2. As expected from previous results (19, 28), the proportion of urease producers in human feces (mean of 14%) tended to be higher than in the rumen (mean of 5.8% excluding the one high value). Only one rumen sample from one animal contained a large number (i.e., 52% of total bacteria) of urease-forming bacteria. Other samples from the same or other animals on the same diet contained considerably fewer urease formers. The reason for the one sample with high proportion is now known, but all urease-forming strains from this sample were presumptively identified as *Succinivibrio dextrinosolvens*, a species found sporadically in very high numbers in cattle fed high-grain diets (7). The results suggest that ureolytic bacteria are present in higher numbers and percentages of total viable bacteria in rumens of cattle fed diets 65, 66, and 67 (average of 8% excluding the high value) as compared to a common hay-grain diet (average of 1%). The reason for the difference is unknown, but diets 65, 66, and 67 are relatively high-grain diets and are unusually low in crude protein. It could be that higher proportions of urease-forming bacteria are selected where rumen ammonia concentrations are low, such as when high-energy-low-nitrogen rations are fed (see J. E. Wohlt, J. H. Clark, and F. S. Blaisdell, Am. Dairy Sci. Assoc. Prog., p. 117-118, 1976, for data on animals used in the present study). On the low-nitrogen diets 65 and 67, the numbers of rumen urease formers were similar with either urea or soybean meal as supplemental nitrogen.

TABLE 2. *Total colony counts and percentage of ureolytic bacteria isolated from rumen contents of cows and human feces using a nonselective rumen fluid roll-tube medium*

Sample	Ration	Total colony counts/g (wet wt)	No. of colonies picked	Ureolytic bacteria (%)
Fecal				
W-1	Unrestricted	6.5×10^{10}	52	19.2
D-1		5.9×10^{10}	36	22.2
D-2		4.2×10^{10}	58	8.6
		3.0×10^{10}	40	7.5
		2.9×10^{10}[a]	61	13.1
W-2		1.2×10^{11}	53	9.4
		1.1×10^{11}[a]	40	15.0
		1.1×10^{11}	47	19.0
Rumen				
3121[b]	65	1.8×10^{9}	50	10
3121	65	9.8×10^{8}	50	14
3022	66	8.4×10^{9}	50	52
2660	66	2.7×10^{9}	50	6
3022	66	1.1×10^{10}	50	8
2818	66	3.0×10^{9}	50	6
2778	67	1.8×10^{9}	50	6
2776	67	1.2×10^{9}	50	4
2549	Hay-grain	3.4×10^{9}	75	0
2549	Hay-grain	3.0×10^{9}	75	0
2-18	Hay-grain	4.6×10^{9}	50	4

[a] Trypticase (0.2%) was added to the isolation medium.
[b] Cow number.

Of the urease-forming cultures from human feces, 62% closely resembled *P. productus* and were not studied further.

The identity and some characteristics of strains studied in detail are shown in Table 3.

The three strains of *S. dextrinosolvens* studied in detail were selected from 20 presumptively identified strains, which represented isolates from six samples from five cows fed diets 65, 66, and 67 but not those from the hay-grain-fed animals. They were typical of the biotypes, fermenting few carbohydrates, i.e., only glucose, maltose, and xylose (8). Fermentation of dextrin and galactose, usually fermented by this type, was not determined.

Two strains of *Treponema* sp. were isolated from a cow fed ration 66, and the third (strain 7) was isolated from a cow fed hay-grain. They were morphologically identical to rumen strains of *Treponema* sp. (*Borrelia*) previously studied (1), except that strain U9 had greater amplitude and a shorter wavelength of coils. They were similar in physiological features to the one strain previously studied (1) in that succinate and acetate were the major fermentation products, but they usually differed in the fermentation of starch, rhamnose, fructose, and lactose. Whether urease formation is typical of rumen or other species of the genus *Treponema* is not known, but this appears to be the first

TABLE 3. *Some characteristics of some strains of anaerobic urease-forming bacteria isolated from the rumen and human feces*[a]

Characteristic	Rumen										Human feces			
	Succinivibrio dextrinosolvens	*Treponema* sp.	*Ruminococcus bromii*	*Coprococcus* sp.	Unknown curved rod	*Butyrivibrio fibrisolvens*	*Peptostreptococcus productus* II	*Butyrivibrio* sp.	*Bacteroides ruminicola*	*Bifidobacterium* sp.	*Ruminococcus albus*	*Clostridium beijerinckii*	*Clostridium innocuum*	*Clostridium* sp.
Amygdalin	•	w–	•	–	–	w	a	–	–	–	w–	a	–	a
Arabinose	•	w–	•	a	–	a	a	w	–	–	–w	a	–	a
Cellobiose	•	wa	•	a	–	a	a	w	a	–	a	a	a	a
Esculin pH	•	–	•	–	–	–	a	–	a	–	–	–	–	w
Esculin hydrolysis	•	+	–	+	+	+	+	+	+	–	+–	+	+	+
Fructose	•	w–	w	a	a	w	a	–	a	a	w	a	a	a
Glucose	w	w–	w	a	a	w	a	–	a	a	a	a	a	a
Glycogen	•	w–	wa	–	–	a	a	–	a	a	–	–	–	–
Lactose	•	–w	•	a	a	w	a	w	a	a	a	a	–	a
Maltose	w	w–	aw	a	a	w	a	w	a	a	–	a	–	a
Mannitol	•	–	•	–	–	–	a	–	–	–	–	–	a	–
Mannose	•	a–	•	–	a	a	a	–	a	–	aw	w	a	a
Melezitose	•	–	•	–	–	–	a	–	w	–	–	–	–	–
Melibiose	•	w–	•	–	a	w	a	–	a	a	–	a	–	a
Raffinose	•	–	•	–	a	–	a	–	a	a	–	a	–	a
Rhamnose	•	–	•	a	–	–	w	–	w	–	–	a	–	a
Ribose	•	–	•	a	–	–	a	–	–	w	–	–	a	a
Salicin	•	–	•	–	–	–	a	–	a	–	–w	a	w	w
Sorbitol	•	–	•	–	–	–	–	–	–	–	–	–	–	–
Starch pH	–	wa	w	–	w	w	a	–	a	a	–	w	–	–
Starch hydrolysis	–+	+	+	–	–	+	+	–	+	+	–	–	–	–
Sucrose	•	–w	•	a	a	w	a	–	a	a	aw	a	a	a
Trehalose	•	–w	•	–	–	–	a	–	–	–	–	a	a	a
Xylose	w	wa	•	a	–	a	a	–	a	a	–	a	–	a
Milk	–	–	–	–	c	c	c	c	c	c	–	–	–	–
Motility	+	+	–	–	+	+	–	+	–	–	–	+	–	+
Gas	–	–	–	+	–	–	–	+	+	+	+	+	+	+
Ferm. acids	Sa (pb)	Sa (f)	La	BAl	Ls bpa	Ba	SAL	Ba	SAl pb	Al	Af	AF bl	LBa	LBa
H_2	–	–	+	+	–	+	+	+	–	–	+	+	+	+
Strains	C18 U13 S29	7 U9 U34	C13 C33	36	U20	36-18	40	C8	28	C40	OT27 OT2 015	L3	D6	W2

[a] None of the strains produced acid from erythritol or inositol, liquefied gelatin, or produced nitrite or indole. Symbols used are as follows: w = acid produced (pH 5.6 to 6.0); a = acid produced (pH 5.5 or below); A, B, L, S, P, F = acetic, butyric, lactic, succinic, propionic and formic acids, respectively; lowercase letters indicate small amounts and those in parentheses indicate variability between strains; – = negative results; • = no growth or very slight growth with no fermentation.

report of urease formation by any spirochete other than *Leptospira*. Some strains of *Leptospira* have a urease that is strongly repressed by ammonia (20).

The three strains identified as *Ruminococcus bromii* were representative of five strains isolated from three samples from two cows on diets 65 and 66. Their characteristics were typical of strains of *R. bromii* isolated from human feces and swine intestine (23). *R. bromii* was not previously known to include ureolytic strains, and the presence of this species in the rumen was not previously established. However, in retrospect, it now seems highly probable that the +C−S group of gram-positive, long-chained cocci isolated as a small proportion of total isolates from many samples of rumen contents by Bryant and Burkey (5) was *R. bromii*. Although not studied in detail, these cocci were inactive toward gelatin, xylose, glucose, cellobiose, and cellulose and did not produce H_2S but rapidly hydrolyzed starch.

Seven strains of motile, curved, gram-negative rods formed a group, but three of these strains selected for detailed study represent three separate species. Strains C8, U20, and 36-18 were isolated from different cows fed diets 65, 66, and 67, respectively, whereas three of the strains not studied in detail came from the same sample as U20 and one came from a sample from another cow fed diet 65. Strain U20 is an unidentified curved rod with monotrichous polar flagellation. It could be assigned to the genus *Succinivibrio*, but it differs from the presently recognized species in many respects. Strain 36-18 has characteristics that would permit its inclusion in *Butyrivibrio fibrisolvens* (3) or *Eubacterium rectale* IIIH (24). Strain C8 appears to be an unidentified species of the genus *Butyrivibrio*. Similar strains have been isolated from human feces and designated Virginia Polytechnic Institute (VPI) group *Butyrivibrio* Z (unpublished data). None of these curved rods studied in detail was *S. ruminantium*, the urease-forming species isolated from the rumen by John et al. (19). In view of the different species designations of the three strains of this group selected for study, the identity of the four strains not studied in detail is conjectural.

Strain 36, representative of three strains isolated from three samples from two cows fed diet 66, appears to be an unknown species of *Coprococcus* (17).

A single isolate, strain 40 from a cow on diet 66, was identified as *P. productus* II (24). This appears to be the first instance of the isolation of this species from the rumen of a mature ruminant, although it is now recognized that the C1 group of John et al. (19) isolated only from rumens of 1-week-old calves belongs to this species.

Strain 28 from a cow fed diet 66 is a typical strain of *Bacteroides ruminicola*, and strain C40 is a *Bifidobacterium* sp., similar to *B. globosum* (26) except in xylose fermentation and quite different from the ruminal urease-producing bifid organism of Gibbons and Doetsch (15).

Among the urease-forming isolates from human feces, the majority of isolates, as expected from previous results (28), were morphologically similar to *P. productus* and were not studied further. The number of strains of this type were 6, 9, 3, and 16 from samples W-1, W-2, D-1, and D-2 (Table 2), respectively. None of the other urease-forming strains described below were isolated from more than one sample.

Three strains (Table 3) from sample W-2 have characteristics of strains of *Ruminococcus albus* isolated from human feces (16). The fecal strains are unlike the usual rumen isolates of this species because almost all rumen strains ferment cellulose, and ammonia is essential as the main N source for growth (25). None of the present fecal strains fermented cellulose in the form of Whatman no. 1 filter paper (9). Also, they grew better than would be expected in the urease detection control medium in which the only source of ammonia is that contaminating the 0.05% each of added Trypticase and yeast extract. The large amount of growth of the fecal *R. albus* strains in this medium (Table 4) as compared to *S. dextrinosolvens*, a species that also required ammonia (or urea) as the major nitrogen source (6), indicates that the fecal *R. albus* uses N other than ammonia. More research, for example, deoxyribonucleic acid homology studies, should be done to determine whether or not these human fecal strains should continue to be considered *R. albus*.

Three urease-forming strains of the genus *Clostridium* were isolated. Strain L3 from sample W-1 is *Clostridium beijerinckii*. Strain W3, also from sample W-1, is morphologically similar to L3 and is tentatively identified as *C. beijerinckii*. Strain D6 from sample D-1 is *Clostridium innocuum* (17).

Single strains of three other species isolated from feces showed strong urease activity on isolation but, contrary to all other urease-forming strains, were negative when retested about 8 months later. The reason for this is not known, but Cook (12) has shown spontaneous loss of an apparently plasmid-coded urease from *S. faecium*. These strains were identified as *Coprococcus catus*, *Fusobacterium prausnit-*

TABLE 4. *Production of NH_4^+ and growth by rumen and fecal ureolytic bacteria when inoculated into the qualitative urease assay medium containing varying levels of Trypticase and yeast extract*

Species and strain[a]	Group[b]	NH_4^+ production (OD)[c]					
		0.05%[d]		0.2%		0.3%	
		0 mM urea	50 mM urea	0 mM urea	50 mM urea	0 mM urea	50 mM urea
Rumen							
S. ruminantium D	1	0 (.70)	+++ (.32)	0 (.90)	0 (.90)	–	–
R. bromii C13	1	0 (.02)	++ (.06)	0 (.15)	+ (.11)	0 (.28)	0 (.27)
R. bromii C33	2	0 (0)	+++ (.08)	0 (.07)	++ (.15)	0 (.20)	+ (.33)
Treponema sp. U9	1	0 (.02)	++ (.05)	0 (.02)	0 (.04)	0 (.02)	0 (.01)
Treponema sp. U34	1	0 (.10)	+++ (.12)	0 (.13)	0 (.29)	0 (.19)	0 (.19)
Treponema sp. 7	1	0 (.05)	+++ (.20)	0 (.25)	0 (.40)	0 (.30)	0 (.46)
S. dextrinosolvens C18	2	0 (0)	+++ (.27)	0 (.06)	++ (.33)	0 (.02)	+ (.34)
S. dextrinosolvens U13	2	0 (0)	+++ (.31)	0 (.21)	++ (.36)	0 (.13)	+ (.44)
S. dextrinosolvens S29	2	0 (.15)	+++ (.39)	0 (.06)	++ (.39)	0 (.04)	++ (.47)
B. fibrisolvens 36-18	2	0 (.62)	+++ (.95)	0 (.96)	++ (1.1)	0 (1.0)	++ (1.1)
Curved rod U20	1	0 (.52)	+ (.47)	0 (.79)	0 (.71)	0 (.85)	0 (.87)
Coprococcus sp. 36	3	0 (.61)	+++ (.42)	0 (.69)	+++ (.60)	0 (1.5)	+++ (1.4)
P. productus II 40	2	0 (.58)	++ (.57)	0 (.61)	+ (1.0)	0 (1.1)	+ (.57)
B. ruminicola 28	1	0 (1.4)	++ (1.3)	0 (2.0)	0 (2.0)	0 (2.0)	0 (2.0)
Bifidobacterium sp. C40	1	0 (.88)	++ (.95)	0 (1.5)	0 (1.4)	0 (2.0)	0 (2.0)
Butyrivibrio sp. C8	1	0 (.63)	+++ (1.0)	0 (.69)	0 (1.0)	0 (1.1)	0 (1.2)
Fecal							
P. productus II 1-1	3	0 (.59)	+++ (.83)	0 (.89)	+++ (.96)	–	–
C. beijerinckii L3	2	0 (.35)	+++ (.72)	0 (1.5)	+ (1.5)	0 (1.1)	+ (1.4)
Clostridium sp. W2	1	0 (.37)	+++ (.75)	0 (1.3)	+ (1.6)	0 (1.3)	0 (1.4)
C. innocuum D6	1	0 (.61)	++ (.58)	0 (1.1)	0 (.95)	0 (.74)	0 (.89)
R. albus D15	3	0 (.62)	+++ (1.1)	0 (2.0)	+++ (1.9)	0 (1.1)	+++ (1.2)
R. albus H OT2	3	0 (.31)	+++ (.47)	0 (.76)	+++ (.71)	0 (.67)	+++ (.68)
R. albus H 015	3	0 (.51)	+++ (.58)	0 (.06)	+++ (.69)	0 (.72)	+++ (.63)

[a] See Table 3 for description of strains, except for strain D, which is *S. ruminantium* (19), and strain 1-1, which is *P. productus* II (28).

[b] Urease formation groups; see text.

[c] Mean optical density (OD) of duplicate cultures at the end of the incubation period.

[d] Trypticase and yeast extract (percentage of each).

zii, and *Streptococcus mitis*. It seems highly unlikely that the latter species would be of any significance in the bowel of humans.

Urease detection medium with added nitrogen. A further experiment was carried out to determine if urease activity (ammonia production from urea) was undetectable in strains when extra Trypticase and yeast extract were added to the medium, as was the case in *S. ruminantium*. The strains fit roughly into three groups (Table 4): (i) ammonia production from urea was not detected when increased nitrogen was added (*S. ruminantium* and 10 other strains); (ii) ammonia production from urea was somewhat depressed with increased nitrogen (six rumen and one fecal strain); and (ii) ammonia production from urea was not depressed with the increased nitrogen used (one rumen and four fecal strains).

The results in Table 4 also show that most species grew very well in the urease detection medium and that very little ammonia was formed by any of these organisms in the medium without urea, even when 0.3% each of Trypticase and yeast extract was present. Species that did not grow well included *R. bromii*, which uses only maltose among the energy sources added (Table 1), and one strain of *Treponema* sp.; however, even with these, urease activity was easily and repeatedly detected.

General discussion. This study shows that many species of anaerobic bacteria include strains that produce urease and confirms previous evidence (28) that indicated that *P. productus* is by far the most numerous urease-forming bacterium in the human bowel. The work also suggests that most strains of this species contain urease. Although urease production was shown to occur in strains of other species tested, we do not know if this enzyme is characteristic of the majority of strains in these species; if previously tested at all, methods adequate for detection have probably not usually been used (28).

Based on studies to date, one cannot generalize on the most numerous urease-forming species in the rumen. The study of John et al. (19) suggested that ureolytic *S. ruminantium*

strains might be quite numerous; however, although there is a small possibility that two or three of the present strains of motile curved rods were *S. ruminantium*, they were not documented among the strains studied in detail. *S. dextrinosolvens* is known to be very numerous in some cattle fed high-grain diets, but this species is usually not found in cattle fed other rations. The next most numerous rumen species found was *R. bromii*, but it is not often a major rumen bacterium.

It seems peculiar that urease-forming strains of *R. bromii*, which usually comprised a much higher proportion of bacteria in human feces than in the rumen (16), were not detected in feces. Perhaps, as is the case with *S. ruminantium* (19), urease formation is not a feature common to most strains of this species. It would be of interest to determine urease activity of additional strains of all the species identified here.

The biological or ecological significance of the urease present in these bacteria, and especially those in which it is apparently strongly repressed by ammonia or other rapidly used nitrogen sources, is not yet known. Further studies on nitrogen nutrition and metabolism and regulation of urease by representative species of the ecosystems of concern are needed.

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LITERATURE CITED

1. **Bryant, M. P.** 1952. The isolation and characteristics of a spirochete from the bovine rumen. J. Bacteriol. 64:325–335.
2. **Bryant, M. P.** 1972. Commentary on the Hungate technique for culture of anaerobic bacteria. Am. J. Clin. Nutr. 25:1324–1328.
3. **Bryant, M. P.** 1974. Genus *Butyrivibrio* Bryant and Small 1956, 18, p. 420–421. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
4. **Bryant, M. P.** 1974. Nutritional features and ecology of predominant anaerobic bacteria of the intestinal tract. Am. J. Clin. Nutr. 27:1313–1319.
5. **Bryant, M. P., and L. A. Burkey.** 1953. Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. J. Dairy Sci. 37:1176–1183.
6. **Bryant, M. P., and I. M. Robinson.** 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. J. Bacteriol. 84:822–828.
7. **Bryant, M. P., I. M. Robinson, and I. L. Lindahl.** 1961. A note on the flora and fauna in the rumen of steers fed a feedlot bloat-provoking ration and the effect of penicillin. Appl. Microbiol. 9:511–515.
8. **Bryant, M. P., and N. Small.** 1956. Characteristics of two new genera of anaerobic curved rods isolated from the number of cattle. J. Bacterial. 72:22–26.
9. **Bryant, M. P., N. Small, C. Bouma, and I. M. Robinson.** 1958. The characteristics of ruminal anaerobic cellulolytic cocci and *Cillobacterium cellulosolvens* n. sp. J. Bacteriol. 76:529–537.
10. **Bryant, M. P., N. Small, C. Bouma, and I. M. Robinson.** 1958. Studies on the composition of the ruminal flora and fauna of young calves. J. Dairy Sci. 41:1747–1767.
11. **Caldwell, D. R., and M. P. Bryant.** 1966. Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. Appl. Microbiol. 14:794–801.
12. **Cook, A. R.** 1976. Urease activity in the rumen of sheep and the isolation of ureolytic bacteria. J. Gen. Microbiol. 92:32–48.
13. **Cook, A. R.** 1976. The elimination of urease activity in *Streptococcus faecium* as evidence for plasmid-coded urease. J. Gen. Microbiol. 92:49–58.
14. **Eller, C., M. R. Crabill, and M. P. Bryant.** 1971. Anaerobic roll-tube media for nonselective enumeration and isolation of bacteria in human feces. Appl. Microbiol. 22:522–529.
15. **Gibbons, R. J., and R. N. Doetsch.** 1959. Physiological study of an obligately anaerobic ureolytic bacterium. J. Bacteriol. 77:417–428.
16. **Holdeman, L. V., I. J. Good, and W. E. C. Moore.** 1976. Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. Appl. Environ. Microbiol. 31:359–375.
17. **Holdeman, L. V., and W. E. C. Moore.** 1974. New genus *Coprococcus*, twelve new species, and emended descriptions of four previously described species of bacteria from human feces. Int. J. Syst. Bacteriol. 24:260–277.
18. **Holdeman, L. V., and W. E. C. Moore (ed.).** 1975. Anaerobe laboratory manual, 3rd ed. Virginia Polytechnic Institute and State University, Blacksburg, Va.
19. **John, A., H. R. Isaacson, and M. P. Bryant.** 1974. Isolation and characteristics of a ureolytic strain of *Selenomonas ruminantium*. J. Dairy Sci. 57:1003–1014.
20. **Kadis, S., and W. L. Pugh.** 1974. Urea utilization by *Leptospira*. Infect. Immun. 10:793–801.
21. **Kaltwasser, H., J. Kramer, and W. R. Conger.** 1972. Control of urease formation in certain anaerobic bacteria. Arch. Mikrobiol. 81:178–196.
22. **Kramer, J., H. Kaltwasser, and H. G. Schlegel.** 1967. Die Bedeutung der Urease Repression für die taxonomische Klassifizierung von Bakterien. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 21:415–423.
23. **Moore, W. E. C., E. P. Cato, and L. V. Holdeman.** 1972. *Ruminococcus bromii* sp. n. and emendation of the description of *Ruminococcus* Sijpestein. Int. J. Syst. Bacteriol. 22:78–80.
24. **Moore, W. E. C., and L. V. Holdeman.** 1974. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. Appl. Microbiol. 27:961–979.
25. **Rogosa, M.** 1974. Genus III. *Ruminococcus* Sijpestijn 1958, 152, p. 525–527. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
26. **Scardovi, V., L. D. Trovatelli, F. Crociana, and B.**

Sgorbati. 1969. Bifid bacteria in the bovine rumen. New species of the genus *Bifidobacterium*: *B. globosum* n. sp. and *B. ruminola* n. sp. Arch. Mikrobiol. 68:278–294.

27. **Umbreit, W. E., R. H. Burris, and J. F. Stauffer.** 1945. Manometric techniques and related methods for the study of tissue metabolism, p. 106. Burgess Publishing Co., Minneapolis.
28. **Varel, V. H., M. P. Bryant, L. V. Holdeman, and W. E. C. Moore.** 1974. Isolation of ureolytic *Peptostreptococcus productus* from feces using defined medium: failure of common tests. Appl. Microbiol. 28:594–599.
29. **Visek, W. J.** 1972. Effects of urea hydrolysis on cell lifespan and metabolism. Fed. Proc. 31:1178–1193.