BACTEROIDES RUMINICOLA N. SP. AND *SUCCINIMONAS AMYLOLYTICA* THE NEW GENUS AND SPECIES

Species of Succinic Acid-Producing Anaerobic Bacteria of the Bovine Rumen

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Several workers (Johns, 1951; Sijpesteijn and Elsden, 1952; Doetsch *et al.*, 1953) have shown that succinic acid is rapidly decarboxylated by washed cells of mixed rumen bacteria to produce propionic acid. The high Q_{CO_2} of this reaction plus the fact that small amounts of succinic acid accumulate in the rumen after an animal is fed (Sijpesteijn and Elsden, 1952) indicate that large quantities of this acid are formed in the rumen.

Several described species of ruminal bacteria produce succinic acid as a major end product of the fermentation of carbohydrate. These include the cellulolytic coccus, *Ruminococcus flavefaciens* (Sijpesteijn, 1951); a cellulolytic rod, *Bacteroides succinogenes* (Hungate, 1950); *Borrelia* spp. (Bryant, 1952); *Succinivibrio dextrinosolvens* (Bryant and Small, 1956*b*), and *Bacteroides amylophilus* (Hamlin and Hungate, 1956).

Detailed studies of groups of bacteria found in the rumen of cows on a variety of rations (Bryant and Burkey, 1953*a*, *b*, *c*) have led to the finding of two previously undescribed groups of succinic acid-producing bacteria. One of these, *Bacteroides ruminicola* n. sp. is often the most numerous group of succinic acid-producing bacteria found.

The present paper deals with some of the characteristics of these bacteria.

METHODS

The methods used in this study were those reported previously (Bryant and Small, 1956*a*) except as outlined below.

The 20 per cent rumen fluid medium for determination of carbohydrates fermented was modified in that the buffer capacity was lowered by incorporation of 0.06 per cent Na_2CO_3 and a gas mixture of 10 per cent CO_2 and 90 per cent N_2 in place of 0.4 per cent Na_2CO_3 and CO_2 gas, and 0.5 per cent trypticase was added. The final pH was determined in the above medium with 1 per cent glucose added. Fermentation acids were determined in cultures incubated for four days in the latter medium modified to contain the higher buffer capacity.

The medium used to determine gas production was of the following percentage composition: trypticase, 1; glucose, 1; cysteine·HCl, 0.05; resazurin, 0.0001; rumen fluid, 20; agar, 2; and double strength minerals 1 and 2 (Bryant and Burkey, 1953*a*), 7.5 each. The medium was adjusted to pH 6.8 and aliquots of 8 ml were placed in 16 by 150 mm tubes. The cotton-plugged tubes were autoclaved at 15 lb for 15 min. The medium to be used was melted in an Arnold steamer for 15 min, placed in a 45 C water bath, and inoculated with 0.2 ml of culture. After mixing and cooling to solidify the agar, the medium was capped with sterile 2 per cent agar. The cultures were incubated for 1 week at 37 C and checked for visible growth and gas splits.

The strains selected for study were isolated using rumen fluid-glucose-cellobiose agar and the method of Bryant and Burkey (1953*a*). Sixteen of the strains had previously been placed in the R-GXCS group (Bryant and Burkey, 1953*a*). This group was composed of strictly anaerobic, nonmotile, gram-negative rods that included long and short to coccoid cells and often showed chains containing long to coccoid cells. They produced acid from glucose, xylose, and cellobiose and hydrolyzed starch, but not cellulose. They were variable in gelatin liquefaction and some strains showed a weak production of H_2S. Ten strains which appeared to be closely related to this group were included. These strains varied from the R-GXCS group in one or more of the following characteristics: did not produce acid from xylose, did not hydrolyze starch, produced a large amount of H_2S, and contained mainly coccoid to oval cells. Four of the strains (23 and

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D strains) were isolated from a Holstein cow fed a ration of alfalfa hay and grain mixture; two (C strains), from the same animal fed the grain mixture only (Bryant and Burkey, 1953*b*); six (GA strains), from two Holstein cows fed fresh alfalfa in the early bloom stage; eight (B_1 strains), from a Holstein cow fed alfalfa hay; and six strains (B strains), from one of two Jersey calves from 13 to 27 weeks of age that were fed alfalfa hay and the grain mixture (Bryant and Small, 1956*c*).

Four strains selected for study were previously placed in the MOR-GS group of motile rods (Bryant and Burkey, 1953*a*). This group included strictly anaerobic, gram-negative, motile organisms that were coccoid to oval to short rod-shaped. Organisms with these characteristics were remarkably homogeneous in other characteristics studied. They fermented glucose and hydrolyzed starch but did not produce H_2S, liquefy gelatin, produce acid from xylose or cellobiose, or hydrolyze cellulose. One of these was isolated from a Holstein cow on a ration of alfalfa hay and grain mixture and three from a heifer on a ration of alfalfa hay, alfalfa pellets, corn silage, and grain mixture.

RESULTS AND DISCUSSION

Bacteroides ruminicola, n. sp. Young cultures of strains of the R-GXCS group and similar bacteria had the following morphological characteristics in common. They were gram-negative. Some cells were lightly stained with heavily stained granules dispersed unevenly throughout the cells or with a bipolar effect. Spores were not observed. No iodine-staining intracellular polysaccharide was observed. Cells had rounded and usually slightly tapered ends. They occurred mainly as singles and pairs but some chain formation was usually found. Observation of large numbers of cells revealed a few branched cells.

The morphology tended to change in older cultures (2 to 3 days of incubation). Cells became swollen and large round bodies were formed. The cells stained less evenly and more intracellular granules were found. Lysis of many cells occurred.

Wet mounts observed with a phase microscope often showed cells evenly dispersed and with no movement indicating they were imbedded in slime. Wet mounts containing nigrosine often showed a few cells with capsules and occasionally a strain would have many large capsules. Neither of these characteristics was a constant occurrence with any one strain.

Most strains studied could be separated into two groups on the basis of morphology in young cultures. With the exception of those listed below, strains were 0.8 to 1.0 μ wide and 0.8 to 30 μ long. The length of most cells was within the range of 1.2 to 6 μ. About half of the cells were oval. A characteristic observation was to find some chains of long to coccoid cells. Some strains were similar to the above group but more regular in length and with few if any coccoid cells. Strains B888-1 and B932-1 were predominantly short rods 1.5 to 3 μ long and strains B610-1 and B903-1 were somewhat longer with most cells 2 to 7 μ long. Strains GA33, GA103, B_14, B_125, B_145, and B742-1 were composed predominantly of coccoid to oval cells 0.7 to 0.8 μ in width. These strains could easily be mistaken for cocci but close examination always revealed some rod-shaped cells up to 4.0 μ in length.

Three-day-old deep colonies in roll tubes were lenticular and 2 to 4 mm in diameter. Surface colonies were entire, smooth, convex, translucent to opaque, and light buff in color. Colonies of most strains were 1 to 2 mm in diameter. Those of strains C104, B903-1, B932-1, B_125, and D36 were 3 to 4.5 mm in diameter. Strains C104, GA17, GA20, GA33, GA103, B_14, and B_149 showed a fluorescent to "frosted glass" appearance when observed by transmitted light.

The appearance of growth in the liquid glucose medium was usually evenly turbid and often showed more or less slimy consistency. At times growth was evident as a slimy to flocculent sediment. The amount of slime produced varied from time to time with the same strain. Some cultures were viscous enough to remain in place when the tube was inverted. Strains GA33, GA103, B_125, B_145, and B742-1 produced a very heavy, even turbidity.

Strains varied in their ability to grow in glucose medium to which yeast extract and trypticase were added in place of rumen fluid. On the basis of visible growth and pH drop after one week of incubation, strains GA33, GA103, B_14, B_125, B_145, B742-1, and D43 grew as well in this medium as in the rumen fluid medium. Other strains showed only slight or no growth in this medium.

All strains grew well at 30 and 37 C but none showed visible growth or lowered the pH of the medium after 1 week of incubation at 22 or 45 C.

All strains were strictly anaerobic. They would not grow in medium in which the resazurin was oxidized.

The final pH in slightly buffered glucose medium was distributed as follows: 1 strain, 4.6; 19 strains, 5.0 to 5.3; and 6 strains, 5.4 to 5.7.

The Voges-Proskauer reaction was inconsistent but usually negative or very weakly positive.

Ammonia production from peptone was determined on some of the strains in a medium of the following percentage composition: trypticase, 1.5; yeast extract, 0.5; glucose, 0.1; resazurin, 0.0001; cysteine·HCl, 0.05; and Na_2CO_3, 0.4. The medium was in equilibrium with CO_2 gas. It was inoculated with one loop of culture, incubated for 1 week, and tested for ammonia production with Nessler's reagent. Strains $B_1$4, GA33, and B742-1 produced ammonia. Strains GA20, $B_1$18, B610-1, B747-1, B903-1, B888-1, and B932-1 did not. However, the latter strains did not grow well in this medium. As rumen fluid contains considerable ammonia, it would be necessary to determine ammonia quantitatively or devise a medium which contained no ammonia but allowed good growth before the question of ammonia production could be answered.

Some physiological characteristics are shown in table 1.

Fermentation acids produced by some of the strains are shown in table 2. All strains produced considerable amounts of succinic and acetic acids. Formic acid production varied from 0 to 26 per cent of the total. Neither of the strains $B_1$4 nor GA20 produced volatile alcohol.

The characteristics of the strains studied indicate a group of closely related organisms. As they are gram-negative, strictly anaerobic, nonsporeforming rods with rounded ends, they belong

TABLE 1

*Some physiological characteristics of Bacteroides isolated from rumen contents**

Subspecies	*B. ruminicola* subsp. *brevis*			*B. ruminicola* subsp. *ruminicola*							
Biotype	1	2	3	1	2	3	4	5	6	7	8
H_2S production	+	−	+	−	w	−	w	−	−	−	−
Gelatin liquefaction	+	+	+	+	+	−	+	+	−	−	−
Starch hydrolysis	+	+	+	+	+	+	+	−	−	−	−
Acid from											
Xylose	−	−	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	−	+	−
Maltose	+	+	+	+	+	+	+	−	−	+	−
Sucrose	+	+	+	+	+	+	+	+	+	−	−
Dextrin	+	+	+	+	+	+	+	−	−	−	−
Inulin	+	+	+	+	+	+	+	+	−	−	−
Xylan	−	−	+	+	+	+	+	+	+	+	+
Gum arabic	+	+	−	−	−	−	−	−	−	−	−
Salicin	+	−	+	+	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+	+	+	+	−
Growth in trypticase-yeast extract medium	+	+	+	−	−	−	+	−	−	−	−
Strains	GA33	B742-1	GA103	GA20	C104	$B_1$28	D43	B747-1	B903-1	B888-1	B932-1
	$B_1$25		$B_1$4	23	GA9	$B_1$49					
	$B_1$45			GA16	$B_1$18	C24					
				D36	$B_1$19	GA17					
				D30	$B_1$38						
				B610-1							

* None of the strains produced catalase, gas, or indole; reduced NO_3; or fermented lactate, trehalose, cellulose, glycerol, mannitol, or inositol. All strains fermented glucose, galactose, fructose, lactose, and cellobiose.

TABLE 2

*Fermentation acids produced in rumen fluid-glucose medium by strains of Bacteroides ruminicola isolated from rumen contents**

Strain	Total Acid (mEq. per 100 ml)	mEq. Per Cent of Total Acid		
		Acetic	Formic	Succinic
GA33	12.33	21.7	10.1	68.3
GA103	12.81	22.5	7.3	68.9
B742-1	10.98	23.5	13.1	60.0
C104	7.76	24.5	25.5	50.9
$B_1 18$	7.56	42.1	21.4	36.4
23	4.56	22.4	25.9	57.7
B610-1	6.80	25.3	25.9	49.6
B747-1	9.36	21.4	0	78.5
B903-1	10.60	18.9	3.4	74.4
B888-1	9.22	23.0	0	73.6
B932-1	9.56	22.8	1.0	71.7

* No appreciable amount of butyric and longer chain volatile fatty acids, propionic or lactic acids were found (less than 3 per cent of total acid).

in the genus *Bacteroides* as described by Kelly (Breed *et al.*, 1957). They differ in several characteristics from all described species of the genus *Bacteroides*.

Although considerable variation occurred between strains, none of the variable characteristics seemed to separate them adequately into more than one well defined species.

BACTEROIDES RUMINICOLA N. SP.

The name *Bacteroides ruminicola* is proposed for all strains shown in table 1. Its characteristics are based on those of type strain 23.

This organism is a gram-negative, nonmotile rod, 0.8 to 1 μ wide and 0.8 to 30 μ long, with slightly tapered, rounded ends. Most cells are 1.2 to 6 μ long. About half of the cells are oval. Irregular granules and bipolar staining are seen in some cells. In old cultures cells tend to swell and lyse. Cells are often encapsulated. Arrangement of cells is as singles and pairs with some chains containing long to coccoid cells.

Deep colonies are lenticular and 2 to 3 mm in diameter. Surface colonies are smooth, entire, convex, opaque, light buff in color, and 1 to 2 mm in diameter.

Growth in glucose liquid medium is turbid with some slime or slimy sediment.

Strict anaerobe.

Growth occurs at 30 and 37 C but not at 22 or 45 C.

Final pH in glucose medium is 5.5.

Fermentation products produced in glucose medium include succinic, formic and acetic acid. Gas is not produced.

Xylose, arabinose, glucose, galactose, fructose, lactose, cellobiose, maltose, sucrose, dextrin, inulin, xylan, salicin, pectin, and esculin are fermented. Lactate, trehalose, cellulose, glycerol, mannitol, inositol, and gum arabic are not fermented.

Starch is hydrolyzed.

Nitrate is not reduced.

Acetylmethylcarbinol, H_2S, catalase, and indole are not produced.

Gelatin is liquefied.

The organism will not grow in a medium in which trypticase and yeast extract are substituted for rumen fluid.

Source: the reticulo-rumen of a cow.

Because of the marked differences between some strains, it is proposed that the species be divided into two subspecies. The first, *Bacteroides ruminicola* subsp. *ruminicola* n. subsp., includes the type strain 23 and seven other biotypes which differ from the type strain in certain physiological characteristics as shown in table 1. In addition to these differences the following variations are apparent within the subspecies. Strain B610-1 of biotype 1 and biotypes 6, 7, and 8 were more regular in cell length and biotypes 5 to 8 produced little or no formic acid (table 2).

It is apparent that biotypes 5 to 8 form a group somewhat different from the other biotypes because of the differences in formate production and their failure to hydrolyze starch or ferment dextrin. However, for the present they will be retained in the first subspecies.

It is proposed that the remaining strains of the species be placed in a second subspecies.

BACTEROIDES RUMINICOLA SUBSP. *BREVIS* N. SUBSP.

Strain GA33 is the type strain. This subspecies differs from *B. ruminicola* subsp. *ruminicola* in the following characteristics. The majority of cells of each strain are coccoid- to oval-shaped and all strains grow well in medium in which trypticase and yeast extract are added in place of rumen fluid. Also, formic acid production (table 2) was intermediate between biotypes 1 to 4 of

the first subspecies which produced large amounts of formate and biotypes 5 to 8 which produced little or none.

Three biotypes are apparent within the subspecies on the basis of characteristics shown in table 1. It should be noted that biotypes 1 and 2 differ from all other members of the species in fermenting gum arabic and not fermenting xylose or xylan, and biotype 3 is very similar to biotypes 1 to 4 of the first subspecies in that the same carbohydrates are fermented.

Several previously described members of the genus *Bacteroides* isolated from rumen contents appear to be closely related to *B. ruminicola*. More complete unpublished data obtained through the courtesy of Dr. S. R. Elsden indicate that group D of the more numerous carbohydrate fermenting bacteria isolated from the rumen of sheep by Wilson (1953) is similar to biotype 1 of *B. ruminicola* subsp. *brevis*. They appear to be identical in morphology, oxygen relations, and temperature range. Carbohydrates fermented were the same except that group D failed to ferment inulin and xylan was not studied. Also, group D fermented rhamnose, raffinose, and grass levan and did not ferment dulcitol, sorbitol, or erythritol. These carbon sources were not included in the present study. Group D grew in a yeast autolysate medium. Fermentation acids produced by group D included acetic, succinic, and lactic acids but growth in the medium used was poor and the amount of acids produced was too small for the estimation of their relative proportions. The colony of Wilson's group D was of tough consistency. This phenomenon was never observed in colonies of any of the strains of *B. ruminicola* but the yeast autolysate medium used in Wilson's study might account for this difference and the toughness of colonies might be explained by slime production which was repeatedly observed in liquid cultures of the present strains. Wilson did not study such characteristics as H_2S, catalase, indole, or gas production; gelatin liquefaction or NO_3 reduction.

B. amylophilus (Hamlin and Hungate, 1956) is closely related to *B. ruminicola* but ferments only starch and maltose and capsules and slime were not observed.

The ruminal cellulolytic bacterium, *B. succinogenes* Hungate (Breed *et al.*, 1957) is closely related to *B. ruminicola* but differs from the latter in fermenting cellulose, and not fermenting galactose, fructose and sucrose.

The ruminal species *B. amylogenes* provisionally placed in this genus by Doetsch *et al.* (1957) is quite different from other ruminal bacteroides. This species is a slender, curved rod that produces a large amount of butyric acid and no succinic acid from carbohydrate.

Carbohydrate-fermenting species of the genus *Bacteroides* from sources other than the rumen have not been characterized as to fermentation products. This makes it difficult to ascertain their relationship to the succinic acid-producing, non-gas-producing ruminal species. It is probable that the succinic acid-producing species should be placed in a genus or subgenus separate from *B. amylogenes* and gas-producing species found in nonruminants. Other workers are currently studying this problem.

The data presented suggest that all strains previously placed in the R-GXCS group (Bryant and Burkey, 1953*a*, *b*, *c*) belong to the species *B. ruminicola* and that some bacteria which were not placed in this group because of vigorous H_2S production and failure to ferment xylose or hydrolyze starch were closely related organisms. The R-GXCS group accounted for from 6 to 19 per cent of total strains isolated on rumen fluid-glucose-cellobiose agar from cattle fed rations as divergent as alfalfa hay, alfalfa silage, wheat straw, and grain mixture only. This represents numbers of 150 to 380 million per ml of rumen contents. In rumen contents of cattle fed alfalfa hay, alfalfa hay plus grain mixture, or alfalfa silage, the R-GXCS group was the most numerous succinic acid-producing bacterium found. When wheat straw was fed, *B. succinogenes* was more numerous and when grain mixture alone was fed, *Succinivibrio dextrinosolvens* (Bryant and Small, 1956*b*) was more numerous.

The R-GXCS group accounted for a significant number of the total starch-hydrolyzing bacteria isolated. Their percentage of the total strains isolated from cattle on different rations was as follows: wheat straw, 64; alfalfa silage, 51; alfalfa hay, 32; alfalfa hay plus grain mixture, 28; grain mixture only, 10 per cent. These data suggest that in rations high in starch and low in fiber, *B. ruminicola* would be of less importance in starch digestion than in rations lower in starch and higher in fiber.

The fact that Hamlin and Hungate (1956) did

not isolate *B. ruminicola* in their studies on starch-digesting bacteria of the rumen is not readily explained, especially since the closely related species, *B. amylophilus*, was isolated. This might be due to differences in the flora of the animals studied or to the difference in the substrates used in the isolation media—glucose and cellobiose in the present study and starch in that of Hamlin and Hungate. Further studies on the effect of different carbon sources in the isolation medium on the species of bacteria cultured from the rumen appear to be in order. It is probable that when glucose and cellobiose are the substrates the same species of cellulolytic bacteria are found (Bryant and Burkey, 1953*a*; Bryant and Doetsch, 1954; Bryant and Small, 1956*a*) as when cellulose is used as substrate (Hungate, 1950, 1957; Sijpesteijn, 1951).

The numbers in which *B. ruminicola* are found in the rumen, their production of products found in the rumen or further metabolized therein, and their fermentation of substances of such quantitative importance in ruminant rations as xylan and starch, indicate the importance of the organisms in the rumen fermentation. The fact that some strains liquefy gelatin, produce NH_3 from peptone, and produce H_2S indicates that they might be of importance in protein metabolism in the rumen.

Nutritional requirements of B. ruminicola subsp. brevis. As a preliminary in attempts to determine factors affecting slime production, a study was made of some of the nutritional requirements of strain GA33. Table 3 shows the composition of the inoculum medium used in this work. Ingredients other than cysteine·HCl and Na_2CO_3 were adjusted to pH 6.7 and autoclaved at 15 lb for 10 min. Sterile solutions of the cysteine·HCl and Na_2CO_3 were then added, and the medium was tubed under CO_2 in 10-ml amounts. The organism was carried in the inoculum medium at 37 C with 0.1 ml transfer daily. Inoculum consisted of one 4 mm loop of a 24-hr culture. Turbidity was measured in 3 to 10 dilutions with a Cenco-Sheard Photelometer with a blue filter.

Figure 1 shows a growth curve for this organism. Maximum turbidity occurred after about 16 hr incubation with a subsequent rapid drop. Microscopic observation indicated that the cells were swelling and lysing as the turbidity dropped. This observation is identical with previous observations by Bryant and Doetsch (1955) on *Bacteroides succinogenes*.

The organism would not grow when casein hydrolyzate or vitamins were deleted from the inoculum medium but grew well when purines

TABLE 3

Inoculum medium for nutritional studies on Bacteroides ruminicola subsp. brevis

	mg/100 ml		*mg/100 ml*
Thiamin·HCl	0.2	Glucose	300
Ca-D-pantothenate	0.2	Casein hydrolyzate*	200
Riboflavin	0.2	Cysteine·HCl	50
Nicotinamide	0.2	Resazurin	0.1
Pyridoxamine·2HCL	0.1	Na_2CO_3	0.4
p-Aminobenzoic acid	0.01	KH_2PO_4	50
Biotin	0.005	K_2HPO_4	50
Folic acid	0.005	$(NH_4)_2SO_4$	100
Cobalamin	0.0005	$MgSO_4 \cdot 7H_2O$	20
Adenine·SO_4	0.5	$CaCl_2 \cdot 6H_2O$	2
Guanine·HCl	0.5	$ZnSO_4$	0.15
Uracil	0.5	$CuSO_4 \cdot 5H_2O$	0.15
Xanthine	0.5	$NaMoO_4 \cdot 2H_2O$	0.15
Thymine	0.5	$CoCl_2 \cdot 6H_2O$	0.15
		$FeSO_4 \cdot 7H_2O$	4

* Nutritional Biochemicals Corporation "vitamin-free" casein hydrolyzate (enzymatic).

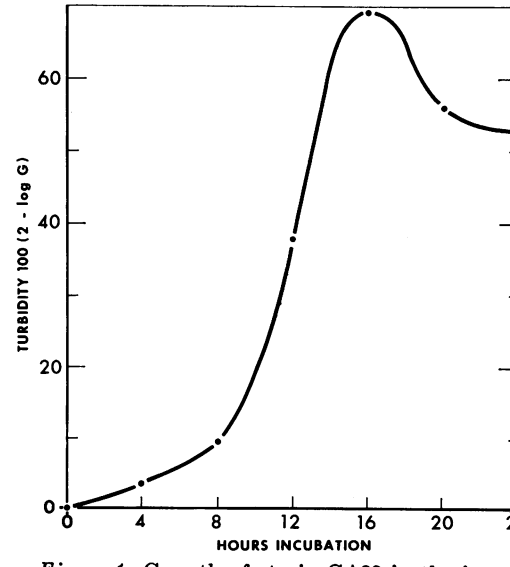

Figure 1. Growth of strain GA33 in the inoculum medium shown in table 3.

and pyrimidines and $(NH_4)_2$ SO_4 were deleted. In media with various mixtures of B vitamins added, it was found that *p*-aminobenzoic acid and biotin were required for growth and the medium with only these vitamins supported as good growth as the medium with nine vitamins.

Amino acids present in amounts similar to those in casein or acid hydrolyzed casein plus tryptophan added to media in place of enzymatically hydrolyzed casein caused a long lag in growth. Turbidity was slight in two days and maximum at about 64-hr. These materials did not delay growth when enzymatically hydrolyzed casein was also present. The factor(s) in enzymatically hydrolyzed casein was dialyzable.

The effect on growth of varying the concentration of CO_2 and Na_2CO_3 added to the medium is shown in table 4. A high level of CO_2 and Na_2CO_3 was required to initiate growth.

Stimulation of growth by enzymatically hydrolyzed casein has been observed in other species of rumen bacteria by Huhtanen (1955). Hall *et al.* (1954) found that mildly acid hydrolyzed or enzymatically hydrolyzed protein contained factors stimulatory to cellulose digestion by washed suspensions of rumen bacteria. Further characteristics of the stimulatory factors suggested that they were peptides.

The genus Succinimonas, n. gen. The four strains studied that were previously placed in the MOR-GS group are quite constant in characteristics. They belong in the family *Pseudomonadaceae* as described in *Bergey's Manual* (Breed *et al.*, 1957) because they are heterotrophic, polarly flagellated, gram-negative, straight rods. They do not belong in any of the genera now placed in this family. Only one genus in the family, *Zymomonas*, Kluyver and Van Niel (1936), includes anaerobes but this genus includes organisms that produce carbon dioxide and ethanol from carbohydrate. As precedence has been established in using fermentation products in the description of the genus *Zymomonas*, it seems best to place the present organisms in a new genus on the basis of their production of large amounts of succinic acid from glucose.

The name *Succinimonas* n. gen. is proposed for anaerobic, nonsporeforming, gram-negative, straight rods with rounded ends that are motile with polar flagella. They ferment carbohydrate with the production of a large amount of succinic acid. The type species is described below on the basis of the four strains studied. Strain B_24 is the type strain.

TABLE 4

*The effect of the concentration of CO_2 and Na_2CO_3 on the growth of strain GA33**

Per Cent			Turbidity—100(2-log G)	
CO_2	N_2	Na_2CO_3	20 hr	44 hr
0	100	0	0	0
5	95	0.03	0	0
10	90	0.06	3	36
50	50	0.30	70	53

* The medium contained ingredients as in the inoculum medium except that purines and pyrimidines, $(NH_4)_2SO_4$, and vitamins with the exception of biotin and *p*-aminobenzoic acid were deleted and the gaseous phase and $NaCO_3$ concentrations were as shown above.

SUCCINIMONAS AMYLOLYTICA N. SP.

This species is a gram-negative, short, rounded end rod to coccoid organism 1.0 to 1.5 μ wide and 1.2 to 3 μ long with most cells oval in shape. Intracellular granules are often present around the periphery of the cells and bipolar staining is evident in some cells. Arrangement of cells is as singles, diplos, and clumps. The organism is motile with polar, monotrichous flagellation. Capsules are not seen.

Deep colonies are lenticular and 0.7 to 1 mm in diameter. Surface colonies are smooth, entire, convex, translucent, light buff in color, and 0.7 to 1.5 mm in diameter.

Growth in glucose liquid medium is evident as relatively light, even turbidity.

Strict anaerobe.

Growth occurs at 30 to 37 C but not at 22 or 45 C.

Final pH in glucose medium is 5.2 to 5.8.

No gas is produced.

Glucose, maltose, and dextrin are fermented. Xylose, arabinose, cellobiose, sucrose, lactose, fructose, glycerol, mannitol, inulin, salicin, esculin, gum arabic, xylan, cellulose, and lactate are not fermented.

Starch is hydrolyzed.

Nitrate is not reduced.

H_2S, catalase, and indole are not produced.

Acetylmethylcarbinol production is variable.

Gelatin is not liquefied.

The organism grows well in medium in which trypticase and yeast extract replace rumen fluid but does not grow in medium with no added bicarbonate and N_2 replacing CO_2 in the gaseous phase.

Products produced by strain B_24 in rumen fluid-glucose medium determined using the methods of Bryant and Doetsch (1954) were as follows: succinic acid, 0.72 mM; acetic acid, 0.09 mM; and a trace of propionic acid per 100 ml of medium. There was an uptake of 0.44 mM of CO_2. No ethanol, formic or butyric and longer chained volatile fatty acids, lactate, methane or hydrogen were detected.

Source: the reticulo-rumen of cattle.

S. amylolytica never accounted for a large number of the total strains isolated from the rumen of cattle fed a variety of rations (Bryant and Burkey, 1953*b*, *c*). None were isolated from animals fed alfalfa silage, alfalfa hay, or wheat straw. They accounted for 4.3 and 1.9 per cent of total isolates when alfalfa hay-grain mixture and grain mixture only were fed. These data indicate that *S. amylolytica* was a larger proportion of total ruminal bacteria when starch in the form of a grain mixture was present in the diet. Unpublished data on animals fed fresh alfalfa, alfalfa hay-corn silage-grain mixture, and clover pasture-grain mixture tend to substantiate this finding. The species accounted for 0, 7, and 6.3 per cent of total isolates, respectively, when these rations were fed. However, none were found in two animals fed a ration of blue grass pasture-grain mixture.

The above data plus the physiological characteristics of the species suggest that one of this organism's main roles in the rumen is the fermentation of starch and its hydrolytic products. However, other starch-hydrolyzing bacteria invariably outnumber *S. amylolytica*. With present methods it is not possible to determine quantitatively the part this organism plays in starch digestion in the rumen.

SUMMARY

A detailed study of the characteristics of 30 strains of bacteria previously isolated from the rumen and placed in two groups on the basis of a few characteristics revealed two new species of nonsporeforming, anaerobic bacteria. *Bacteroides ruminicola* n. sp. is a nonmotile organism that ferments a wide variety of carbohydrates and produces succinic and acetic acids and variable amounts of formic acid but no gas in rumen fluid-glucose medium. The species is divided into two subspecies, *B. ruminicola* subsp. *ruminicola* n. subsp. and *B. ruminicola* subsp. *brevis* n. subsp., on the basis of morphology and growth requirements. The second subspecies is shorter with most cells occurring as coccoids and ovals and grows well with trypticase and yeast extract substituted for rumen fluid. The first subspecies is divided into eight biotypes on the basis of H_2S production, gelatin liquefaction, starch hydrolysis, and carbohydrates fermented. The second subspecies is divided into three biotypes on the basis of H_2S production and carbohydrates fermented. This species is often the most numerous succinic acid-producing, starch-hydrolyzing and xylan-fermenting bacterium isolated from the rumen.

A strain of *B. ruminicola* subsp. *brevis* requires CO_2, biotin, *p*-aminobenzoic acid, and unknown factors in enzymatically hydrolyzed casein for optimum growth in a medium containing minerals, cysteine·HCl, and glucose.

Succinimonas amylolytica n. sp. is the type species of the new genus *Succinimonas* in the family *Pseudomonadaceae*. This genus includes anaerobic, nonsporeforming, gram-negative, straight rods with rounded ends that are motile with polar flagella and ferment carbohydrate with the production of a large amount of succinic acid. The four strains studied were very similar in characteristics and all belong to the type species. The species is composed of short rod to coccoid-shaped, monotrichous organisms that ferment starch and its hydrolytic products. In addition to succinic acid production, a small production of acetic acid and a gross uptake of CO_2 was found after growth in rumen fluid-glucose medium. This species is found among the predominant bacteria of cattle fed rations containing grain mixtures in addition to roughage.

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