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Characterization of the Predominant Bacteria Occurring in the Rumen of Goats (*Capra hircus*)[1]

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A total of 44 strains of bacteria were isolated from rumen contents of the goat. Based on morphology, Gram stain, anaerobiosis, motility, and fermentation end products, they were grouped into 11 different types. For each type, all or representative strains were characterized in detail. The type, number of strains characterized over total number of strains, and identification were as follows: type 1, 6/21, atypical *Butyrivibrio fibriosolvens*; type 2, 6/9, atypical *Butyrivibrio fibrisolvens*; type 3, 3/3, genus uncertain; type 4, 2/2, genus uncertain; type 5, 3/3, *Streptoccous bovis*; type 6, 1/1, *Butyrivibrio fibrisolvens*; type 7, 1/1, *Bacteroides ruminicola* subsp. *ruminicola*; type 8, 1/1, *Bacteroides ruminicola* subsp. *brevis*; type 9, 1/1, family *Peptococcaceae*, genus uncertain; type 10, 1/1, genus *Bacteroides*; type 11, 1/1, genus *Bacteroides*. About 70% of the isolated strains were classified as *Butyrivibrio*, which is quite high compared with previous studies in cattle on similar rations. Of the 30 strains listed as type 1 and 2, the 12 studied further were characterized as atypical *Butyrivibrio fibrisolvens*, which differed from the species description primarily by their inability to hydrolyze starch and lack of gas production.

The goat has been used extensively as an experimental animal in studies on the physiology of ruminants (31); however, very little information is available on the rumen fermentation in this species. Comparison of forage digestibility between goats, sheep, and cattle has not revealed any major differences (2, 24). It has been suggested that goats would be well suited for evaluating forages since they are hardy animals requiring less feed than cattle, and they would be preferable to sheep on the basis that they can also be used to measure milk production and composition.

Several quite thorough descriptive studies on the ciliate protozoa occurring in the rumen of goats have been reported (3, 17, 18), and no species have been observed that are considered to be specific to the goat rumen. Dogiel (17) found that the number of different protozoal species observed in goats was less than half the number in cattle and sheep. In a subsequent study, however, additional species were established in the goat by inoculation with bovine rumen contents (18).

Aside from the species *Eubacterium fissicatena*, which was isolated from the rumen of goats and degrades the ribityl chain of riboflavin hydroxyethylflavin (37), the authors are not aware of any studies on rumen bacteria of the goat. However, Sharpe et al. (34) were able to demonstrate a relatively high titer of agglutinating antibodies in goat serum against strains of anaerobic bacteria isolated from bovine rumen contents. The predominant rumen bacterial species occurring in cattle and sheep have been fairly well documented (23). If data on digestibility and rumen metabolism in the goat are to be interpreted as similar to these values in cattle and sheep, some knowledge of the rumen bacteria in the goat is needed. The present study was undertaken to isolate and characterize predominant species of goat rumen bacteria.

MATERIALS AND METHODS

Rumen ingesta was obtained through a permanent rumen fistula from a French Alpine goat, housed at Michigan State University. The animal was fed once daily a maintenance ration consisting of corn cob pellets (45%), 17% (crude protein) dehydrated alfalfa meal (35%), rolled oats (12.6%), molasses (5%), urea (0.4%), and a mineral and vitamin mix (2%). The sample was obtained 1 h before feeding, stored under anaerobic conditions on ice, and transported to Wooster. Approximately 4.5 h elapsed between sampling and processing. Microscopic examination of a portion of the sample that was warmed to 38°C in the incubator indicated that the protozoa were still quite motile. Isolations were made from 40% rumen fluid-glucose-cellobiose-agar (RGCA) medium (7). The anaerobic cultural tech-

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niques, media preparation, and isolation procedures have been previously described (15).

Colonies were picked at random from roll tubes containing 10^{-7}, 10^{-8}, and 10^{-9} g of rumen contents after 20 to 120 h of incubation at 38°C. From a total of 55 colonies picked and stab-inoculated into RGCA slants, 47 were viable. Culture purity of these isolates was established by reisolation through roll tubes. Four or more colonies were picked from the tube with the highest dilution showing growth. If the bacteria from all colonies were identical in morphology under the phase microscope, the culture was presumed to be pure. One of these colonies was then maintained as the stock culture. The methods used for characterization of these bacteria have been reported previously (14, 15).

Organic acid fermentation products were determined by silica gel chromatography (E. G. Linke, M.S. thesis, The Ohio State University, Columbus, 1952). Production of ethanol was estimated qualitatively by gas chromatography (15).

Optical density was measured at 600 nm on a Spectronic-20 colorimeter (Bausch and Lomb, Inc., Rochester, N. Y.).

RESULTS AND DISCUSSION

On the basis of morphology, Gram stain, anaerobiosis, and organic acid end products, goat rumen bacteria were classified into 11 different types (Table 1). Three of the original cultures were lost during storage, so that only 44 cultures are listed in the table. Types 1 and 2 comprised the majority of the isolates, 68%, and were similar in all respects, except for the production or uptake of acetate and the amount of formate produced. Considerable variation was noted among the nine remaining types, six of which were represented by single cultures.

Representative strains of the first four types, all anaerobic, gram-negative, motile curved rods, were selected for more detailed characterization, and these data are presented in Table 2. Most of the 21 strains of type 1 were similar to E44a; however, E21c was chosen for additional characterization studies because it produced lesser amounts of butyrate and small amounts of succinate. It was included in type 1 on the basis of uptake of acetate and low formate production. Six of the nine strains in type 2 resembled E25b, whereas E9a and E46a showed variation in the amount of butyrate, formate, and lactate produced. Strains E26f and E20a were similar to the other strains in types 3 and 4, respectively.

Based on the characteristics presented in Table 2, both type 1 and 2 would appear to fit the genus *Butyrivibrio*. Only one species, *B. fibriosolvens*, has been assigned to the genus, and the present isolates differ from the species description in that very heavy growth without gas splits was observed in glucose agar shake tubes (10). In addition, starch was not hydrolyzed (except to limit dextrins by E46a) and pectin was not fermented, both of which are usually fermented by this species (11). Although the slight amount of succinate produced by E21c would normally exclude this strain from the species *B. fibriosolvens* (11), Lee and Moore (27) and Abou Akkada and Blackburn (1) have detected production of small amounts of this acid in culture medium from *Butyrivibrio* fermentations. The Virginia Polytechnic Institute *Anaerobe Laboratory Manual* has included those *Butyrivibrio* strains producing small amounts of succinate in the species *B. fibrosolvens* (21).

Four additional type 1 strains and three type 2 strains did not produce gas splits in glucose agar or hydrolyze starch. All seven of these additional strains grew well in Trypticase-yeast extract medium; however, only two strains (type 2) would grow in the complete medium. These characteristics agree quite closely with those of the strains listed in Table 2.

TABLE 1. *Preliminary classification of goat rumen bacteria*

Type	Morphology	Motility	Gram stain	Anaerobiosis	Organic acid end products[a]	No. of strains	% of total
1	Curved rod	+	−	+	B, -a, f, L	21	47.7
2	Curved rod	+	−	+	B, a, F, L	9	20.4
3	Crescent	+	−	+	b, a, f, L	3	6.8
4	Curved rod	+	−	+	b, a, f, L	2	4.5
5	Streptococcus	−	+	−	L	3	6.8
6	Short rod	−	−	+	B, -a, f, L	1	2.3
7	Pleomorphic rod	−	−	+	P, A, S	1	2.3
8	Coccus to rod	−	−	+	P, A, f, S	1	2.3
9	Streptococcus	−	Var.[b]	+	b, A, f, l	1	2.3
10	Pleomorphic rod	−	−	+	s, l	1	2.3
11	Rod	−	−	+	a, l, e	1	2.3

[a] Small letters, <1 mmol per 100 ml of medium; capital letters, >1 mmol per 100 ml of medium. B, Butyric and higher acids; P, propionic, A, acetic, F, formic, S, succinic, L, lactic, E, ethanol.

[b] Gram variable.

TABLE 2. *Characteristics of representative strains of anaerobic, gram-negative motile curved rods isolated from goat rumen contents*[a]

Characteristics	Type						
	1		2			3	4
	E21c[b]	E44a	E9a	E25b	E46a	E26f	E20a
Cells							
Length (μm)	1.2-2.5	1.0-2.5	1.0-2.5	1.0-2.5	1.5-2.5	1.0-4.0	1.0-1.5
Width (μm)	0.5	0.4	0.4-0.5	0.5	0.4-0.5	0.6-0.7	0.5
Starch hydrolysis	−	−	−	−	∓	+	+
Final pH, glucose	5.6	5.6	5.6	5.6	6.1	6.1	5.6
Gelatin liquification	−	−	−	−	+	−	−
Gas production	−	−	−	−	−	+	−
Acid from:[c]							
Xylose	+	+	+	+	+	+	−
Esculin	+[w]	+	+	+	+[w]	−	+[w]
Xylan	+	+	+	+	+	+	−
Inulin	−	−	−	v	−	+	v
Mannitol	−	−	−	−	−	+	−
Lactose	+	+	+	−	−	+[w]	+
Galactose	+	+	+	−	+[w]	+	+
Rhamnose	−	−	−	−	−	+[w]	−
Growth in Trypticase-yeast extract medium	1.36(20)[d]	1.28(18)	1.34(20)	0.87(22)	0.16(60)	1.28(18)	0.48(19)
Growth in complete medium[e]	0.24(75)	0.22(118)	0.22(55)	1.24(21)	0.03(55)	0.42(96)	0.60(32)
Plus 10% rumen fluid	0.89(20)	1.01(20)	0.94(22)	1.22(18)	0.51(52)	1.40(20)	1.13(22)
Organic acid end products (mmol/100 ml)[f]							
Butyric and higher	0.66	1.71	1.19	1.72	0.75	0.15	0.11
Propionic	−0.05	−0.02	0.03	−0.02	0.10	0.15	0.01
Acetic	−0.48	−0.89	0.68	0.57	0.42	0.90	0.17
Formic	0.40	0.88	1.09	2.45	2.00	0.46	0.34
Succinic	0.11	0	0	0	0	0.17	0
Lactic	5.09	4.37	6.06	1.56	1.40	3.79	7.56

[a] None of the strains produced H_2S (could not be determined for E20a) or indole, reduced nitrate, digested cellulose, or produced acid from pectin, glycerol or lactate. All strains produced acid from glucose, cellobiose, dextrin, fructose, arabinose, sucrose, maltose and salicin. In addition to growth at 38°C, strains E21c, E44a, E9a, and E25b grew at 22°C, 30°C, and 45°C; strains E20a and E46a grew at 30°C.

[b] Strains.

[c] +, Strong acid production, a decrease of 0.3 pH units or more. +[w], weak acid production, a decrease of 0.15 to 0.3 pH units; −, no acid production, a decrease of less than 0.15 pH units; v, variation from + to − between replicate trials.

[d] Values are the increase in optical density at 600 nm. The figure in parentheses indicates the hours required to reach maximum optical density.

[e] Defined medium plus acid hydrolysate of casein, hemin, and volatile fatty acids (32), containing 0.5% glucose as an energy source.

[f] Produced in a 20% rumen fluid−0.5% Trypticase−1% glucose broth medium (10). None of the strains produced ethanol.

In view of the acknowledged heterogeneity of the genus *Butyrivibrio* (11), it is probably best to classify these cultures as atypical strains of *Butyrivibrio fibrisolvens*. Moore et al. (30) have recently emended *Butyrivibrio* and described a new species, *B. crossotus*, isolated from human feces. However, characteristics of this new species differ markedly from the present *Butyrivibrio* strains. Criteria such as guanine plus cytosine content of the cellular deoxyribonucleic acid and possibly base sequence homologies (13) might be useful in deciding whether additional species are warranted.

Characteristics of a type 3 isolate, strain E26f, are listed in Table 2. Based on morphology, end products, and fermentation of mannitol, the organism would appear to resemble *Selenomonas ruminantium* (5); however, it differs in not fermenting esculin or producing propionate, having a relatively high minimum pH for growth, and in fermenting xylan. These latter characteristics are similar to those described for strain B-385, which is morphologically somewhat similar to *Selenomonas* but physiologically similar to *B. fibrisolvens* (5). Strain B-385-like organisms exhibiting polar tufts of flagella are now placed in the genus *Butyrivibrio* as emended (30), from which E26f would be excluded on the basis of negligble butyrate production and the fermentation of mannitol. The two additional strains of type 3 did not produce H_2S, hydrolyzed starch, produced gas, grew well in Trypticase-yeast extract medium, and produced fermentation prod-

ucts similar to E26f. Attempts were made to determine the type of flagellation for strain E26f and the two similar strains by electron microscopy; however, results were inconclusive. All three strains appeared to produce a capsular-like material which resulted in very poor resolution. This was overcome to some degree by growing the cells in a complete medium (32) with half the regular concentration of minerals and harvesting after 14 to 16 h of growth. The following conclusions were reached from observation of these preparations by electron microscopy: (i) cells are crescent-shaped; (ii) no flagellar tuft or tuft base was observed on any of the cells; (iii) a few extremely long, monotrichous type, unattached flagella were observed; and (iv) indications of one or possibly two flagellar stubs were observed in the polar region of several cells. With the information available, identification of these strains is questionable. Therefore, they have simply been listed as genus uncertain. Additional studies, in which butyric acid production was determined in various media might help to determine their possible relationship to the genus *Butyrivibrio*.

The type 4 strain, E20a, was quite small and differed markedly by its inability to grow in the test medium for H_2S production. This strain was unable to ferment xylose or xylan, and 80% of the total acid produced was lactic. Electron microscopy revealed the presence of a single polar to subpolar flagellum. Although there was very low production of butyric acid and lack of gas splits, most characteristics of this strain are similar to the genus *Butyrivibrio*. The second strain of type 4, E10a, was similar in size, hydrolyzed starch, did not produce gas splits, and grew better in complete medium than in Trypticase-yeast extract medium. For strain E10a, 91.5% of the acid produced was lactic and less than 1% was butyric. Some strains of *Butyrivibrio* have been found to produce high lactic and little butyric acid in certain media, particularly media without rumen fluid (6, 19, 27). If studies did indicate that these type 4 strains might be included with *Butyrivibrio*, their variation in butyric acid production and inability to grow in the H_2S production medium (presumably an inhibiting effect of ferric ammonium citrate) would set them apart as a unique species or subspecies. Genus designation for these two strains is uncertain.

The three strains classified as type 5 were nonmotile, gram-positive spherical-to-ovoid cells about 1.0 μm in diameter, occurring singly, in pairs, and in short chains. All three strains produced orange pigment on solid media, were facultative anaerobes, and produced only lactic acid from the fermentation of glucose. Based on these criteria, they were readily identified as strains of *Streptococcus bovis* (11, 22).

Strain E38f, type 6, was originally classified as a separate type on the basis of cell morphology. All characteristics of this strain (Table 3) would coincide with the species description for *Butyrivibrio fibrisolvens*; however, the majority of cells were spindle shaped, measuring about 0.5 by 1.0 μm, occurring singly and in pairs, and short chains. A few larger crescent-shaped cells could always be observed, and since variation in cell size and morphology is not uncommon for this species (9, 11), E38f might be a *B. fibrisolvens*.

Strain E40a, type 7, is a gram-negative, nonmotile short rod, with many pleomorphic cells generally visible. These factors, along with the production of large amounts of succinic acid, suggest a close relationship to the genus *Bacteroides*. Stimulation of growth in complete medium by volatile fatty acids, ability to grow with ammonia as the sole nitrogen source, an absolute requirement for hemin, plus the other characteristics measured, allow ready identification of strain E40a as *Bacteroides ruminicola* subsp. *ruminicola* (11).

Classification of strain E42g as a separate morphological type, type 8, was based on the observation that at least 99+% of the cells were coccoid to oval, measuring about 0.5 to 0.6 μm. However, with more careful microscopic examination occasional rod forms were observed, especially in older cultures. On this basis and because of the characteristics listed in Table 3, strain E42g has been identified as *Bacteroides ruminicola*. Since this strain was hemin independent, it belongs in the subspecies *brevis* (8). It differs from the subspecies description in not hydrolyzing starch or producing acid from dextrin or maltose (10, 11); however, a previously described strain of *B. rumincola* subsp. *brevis* had similar characteristics (14).

Strain E45b belongs in the family *Peptococcaceae* because it is an anaerobic, nonmotile, gram-variable-to-gram-positive coccus which occurs in long chains. Of the four genera in this family, this strain appears most closely related to the genus *Ruminococcus*, particularly since the genus has been emended to include noncellulolytic species (29). Morphology, physiological characteristics, and an obligate requirement for volatile fatty acids are all compatible with the genus description; however, the production of butyrate (or higher acids) as a major end product would exclude it from this genus. Determination of deoxyribonucleic acid base

TABLE 3. *Characteristics of the less-frequently occurring morphological types of bacteria isolated from goat rumen contents*[a]

Characteristic	Type					
	6	7	8	9	10	11
	E38f[b]	E40a	E42g	E45b	E54i	E55c
Cells						
Length (μm)	1.0–2.5	1.0–30.0	0.5–3.0		1.0–2.5	1.0–3.0
Width (μm)	0.5–0.6	0.7–1.0	0.5–0.8	0.5	0.6	0.5
Gram stain	–	–	–	Var.[c]	–	–
Motility	+	–	–	–	–	–
Starch hydrolysis	+	+	–	–	–	–
Final pH, glucose	5.6	5.6	5.7	6.0	6.3	6.4
Gelatin liquifaction	–	–	+	–	–	–
Gas production	+	–	–	+	–	–
Acid from:[d]						
Glucose	+	+	+	+	–	–
Galactose	+	+	+	+[w]	–	+[w]
Xylose	+	+	+	+	–	+[w]
Dextrin	+	+	–	–	–	+[w]
Fructose	+	+	+	–	–	–
Arabinose	+	+	+	+	–	–
Esculin	+[w]	+	v	–	–	–
Xylan	+	+	+	+	–	v
Pectin	–	+[w]	+[w]	–	–	–
Inulin	+	+	+	–	–	–
Maltose	+	+	–	–	–	–
Lactose	+	+	+	+[w]	–	–
Salicin	+[w]	+[w]	v	–	–	–
Growth in Trypticase-yeast extract medium	0.96(18)[e]	0.30(17)	1.16(22)	0.26(20)	0.12(82)	0.02(90)
Growth in complete medium[f]	1.41(18)	1.54(23)	1.32(34)	1.00(23)	0.03(44)	0.03(17)
Minus hemin	1.23(20)	0.03(17)	1.36(45)	1.02(24)	0.04(118)	0.04(118)
Minus VFA	1.42(20)	0.62(117)	1.00(168)	0.02(32)	0.01(20)	0.02(36)
Minus casein hydrolysate	1.24(24)	1.24(36)	1.48(44)	0.98(108)	0.02(168)	0.04(24)
Plus 10% rumen fluid	1.30(20)	1.40(20)	1.24(30)	0.80(30)	0.26(105)	0.26(102)
Organic acid end products (mmol/100 ml)[g]						
Butyric and higher	1.25	−0.02	0.02	0.91	−0.11	0.01
Propionic	−0.02	1.38	1.50	0.02	0.03	0.01
Acetic	−0.30	2.31	1.37	1.61	−0.06	0.55
Formic	0.41	0	0.32	0.57	0	0
Succinic	0	1.52	1.58	0	0	0
Lactic	4.85	0	0	0.24	0.41	0

[a] Morphology of the different strains is shown in Table 1. All strains are anaerobic, do not produce H_2S or indole, reduce nitrate, digest cellulose, or produce acid from mannitol, glycerol, lactate, or rhamnose. All strains produced acid from cellobiose and sucrose.

[b] Strain designation.

[c] Gram variable.

[d] See footnote *b*, Table 2, for explanation of legend.

[e] Values are the increase in optical density at 600 nm. The figure in parentheses indicates the hours required to reach maximum optical density.

[f] Defined medium plus acid hydrolysate of casein, hemin, and volatile fatty acids (VFA) (32), containing 0.5% glucose as an energy source.

[g] Produced in a 20% rumen fluid-0.5% Trypticase-1% glucose broth medium (10). Strain E55c produced a small amount of ethanol.

composition would probably be required to adequately identify this strain.

Since strain E54i is a gram-negative, anaerobic, nonmotile, pleomorphic rod which does not form spores, it has been classified in the family *Bacteroidaceae*. This strain did not ferment any of the carbohydrates tested (Table 3), based on the criteria of a drop of >0.15 pH units. Slow

growth occurred in RGCA slants and a slight increase in optical density was measured with glucose and starch substrates. The only end product measureable from glucose was lactic acid, whereas small amounts of succinate and lactate were present in starch broth fermentations. Observation of this strain with the phase microscope showed a morphology that was strikingly similar to *B. amylophilus* (20). Gram-stained smears also showed internal gram-positive granules for many cells. Based on morphology and some succinate production, it is suggested that strain E54i should be classified in the genus *Bacteroides*. Presumably this strain either does not ferment carbohydrates, or only weakly so, or its nutritional requirements have not been met by the media used.

Strain E55c also is a weak carbohydrate fermenting species, producing only acetic acid and small amounts of ethanol as end products of glucose fermentation. In addition, some lactic acid was produced in the fermentation of cellobiose. Morphologically it is an anaerobic, nonmotile, nonsporeforming, gram-negative straight rod with rounded ends, which would place it in the family *Bacteroidaceae*, and probably most closely associated with the genus *Bacteroides* on the basis of end products. Its fermentation and biochemical reactions differ from any of the described species of this genus (11).

Although the total number of isolates studied was limited, the extremely high proportion of *Butyrivibrio* species (70%), types 1, 2, and 6, was most unusual. Previous investigations with cattle have reported the following percentages for typical plus atypical *Butyrivibrio* species on different rations: all roughage, 38 and 42%; 90% roughage, 50%; 63% roughage, 32%; 44% roughage, 25%. This would contrast with percentages of 0 to 12.5 on rations containing at least 90% concentrate (12, 25, 26, 35, 36, 38).

As mentioned previously, considerable variation has been observed with the genus *Butyrivibrio*; however, the 12 type 1 and 2 strains studied in detail here were almost unique. In Table 4, these 12 strains plus strain E38F (type 6) are compared with strains of *Butyrivibrio fibrisolvens* isolated from various hosts and locations throughout the world. The inability of the strains from the goat to hydrolyze starch, ferment pectin, or produce gas sets them apart from previously studied strains. The possibility exists that the present isolates may produce gas, but in amounts too low to split the agar in agar shake tubes, even though heavy growth was visible. Several investigators have reported the occurrence of presumptively identified atypical *Butyrivibrio*-like organisms; however, none of these have been studied in detail (12, 35, 36, 38). Slyter et al. (35) did report that some of their atypical *Butyrivibrio fibrisolvens*-like strains did not produce gas.

The scope of the present study does not allow any overall conclusions regarding the similarity of the microbial population between goats and other domestic ruminants. On the other hand, only two strains of *Bacteroides ruminicola*, three strains of *Streptococcus bovis*, and a single strain of *Butyrivibrio fibrisolvens* were readily classified as species typical with those occurring in cattle and sheep. Further studies with additional animals on different rations would be of considerable interest.

TABLE 4. *Comparison of Butyrivibrio strains isolated from various hosts and locations*

Source	Host	Location	Total no. of strains	% Hydrolyzing starch[a]	% Fermenting pectin	Gas production[b]	Lactate production[b]
Bryant & Small (9)	Cattle	Md., U.S.A.	48	50	ND[c]	2/2	2/2
Shane et al. (33)	Sheep	South Africa	19	100	100	19/19	11/19
Margherita & Hungate (28)	Zebu cattle	Kenya	6	50	83	5/5	5/5
Hungate (23)	Cattle	Calif., U.S.A.	12	66	58	12/12	7/12
Dehority (14)	Cattle	Ohio, U.S.A.	5	100	ND	5/5	5/5
Dehority (15)	Cattle	Ohio, U.S.A.	4	75	100	4/4	4/4
Van Gylswyk & Roche (39)	Sheep	South Africa	10	100	100	10/10	10/10
Dehority (16)	Reindeer	Alaska, U.S.A.	8	50	ND	ND	3/8
Brown & Moore (4)	Rabbit, human, & horse feces	Va., U.S.A.	5	100	ND	ND	ND
Present study	Goat	Mich.,U.S.A.	13	8	0[d]	1/13	13/13

[a] Strains hydrolyzing starch to limit dextrins were considered as negative for starch hydrolysis.
[b] Positive strains/total strains tested.
[c] ND, Not determined.
[d] Determined on only 6 of the 13 strains.

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