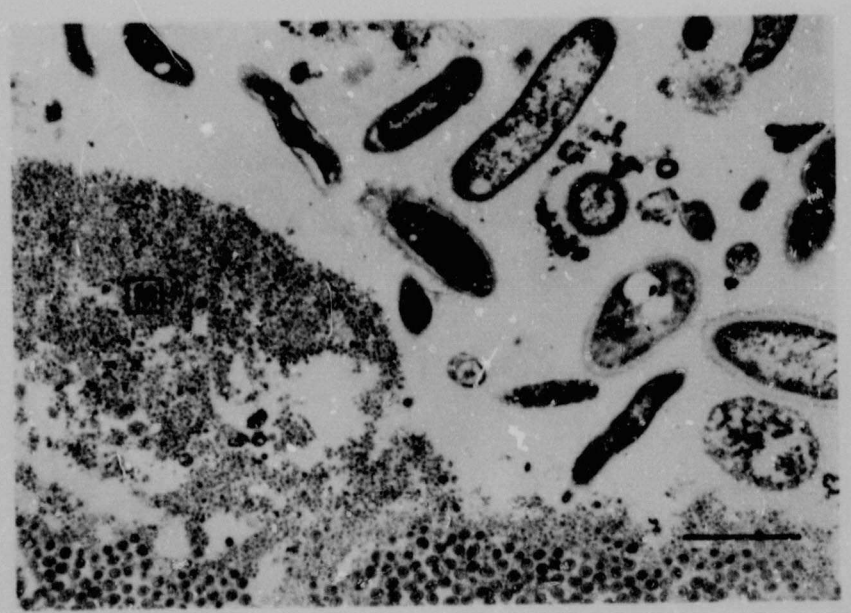

Figure 7. TEM section of microvesicular layer (M) separating the brush border of the epithelium and the mucous gel in the rabbit cecum. Bar = 1 µm

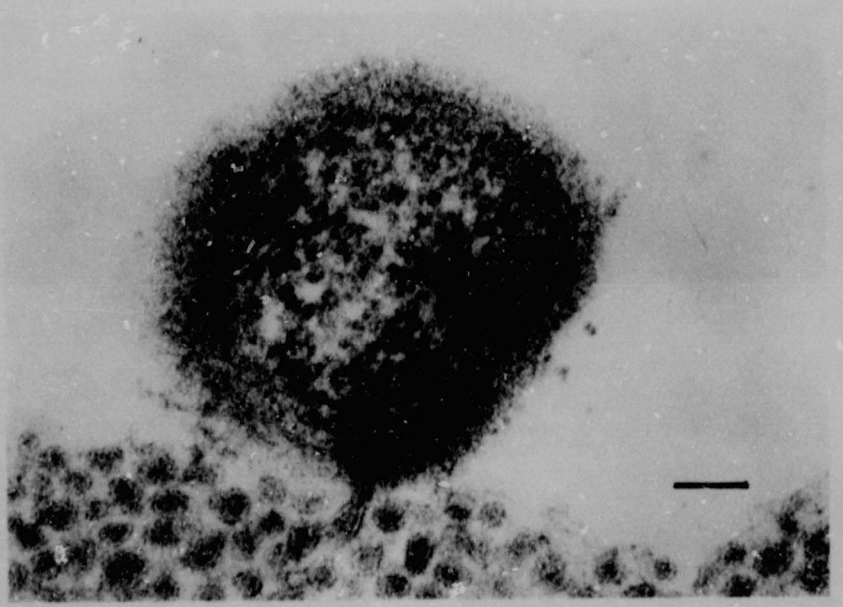

Figure 8. TEM section of a bacterium in close contact with the microvesiclar layer of rabbit cecum. Bar = 0.1 µm

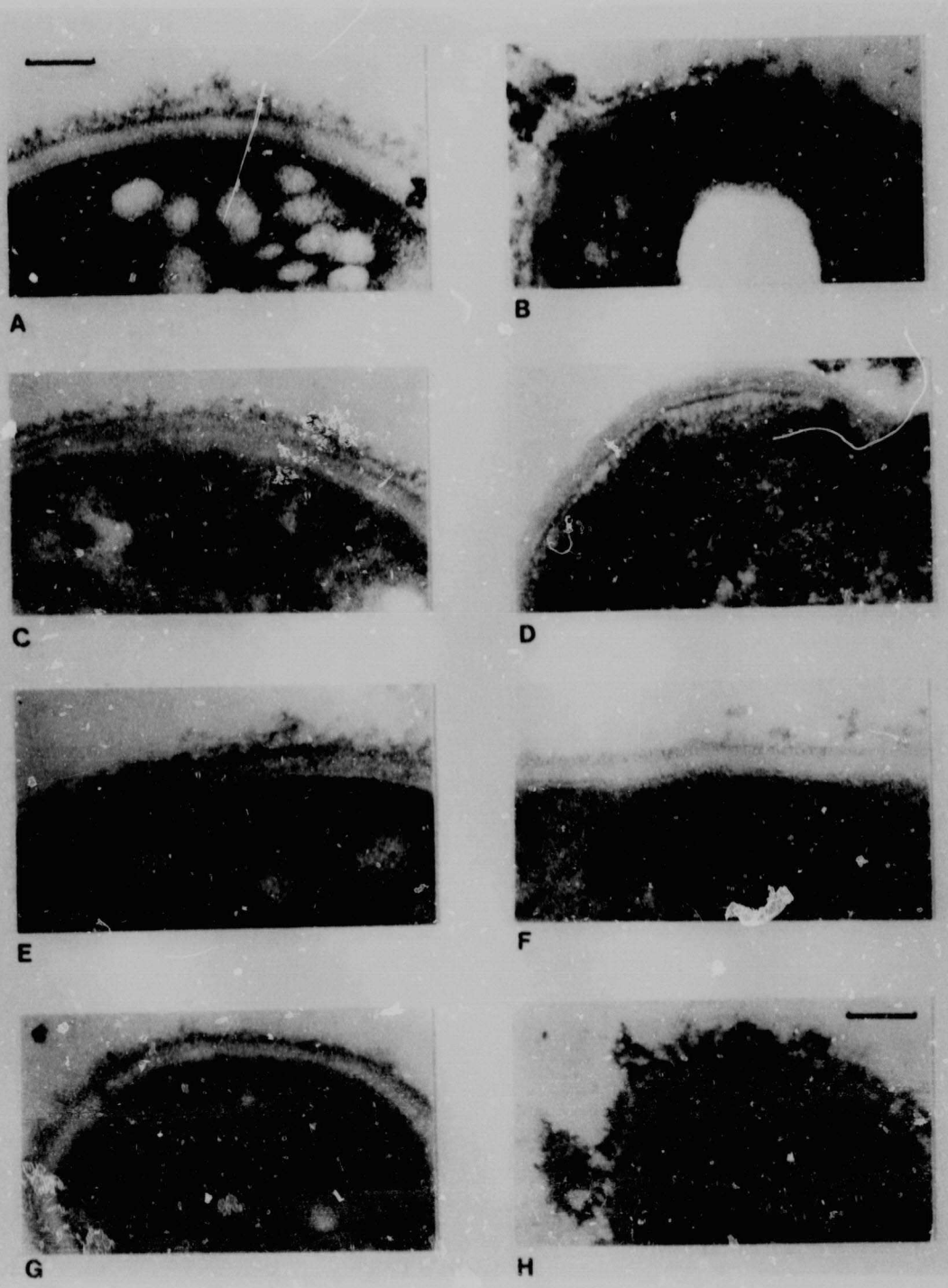

Figure 9. Classical Gram positive cell walls from TEM sections of cecal mucus. Bar = 0.1 μm

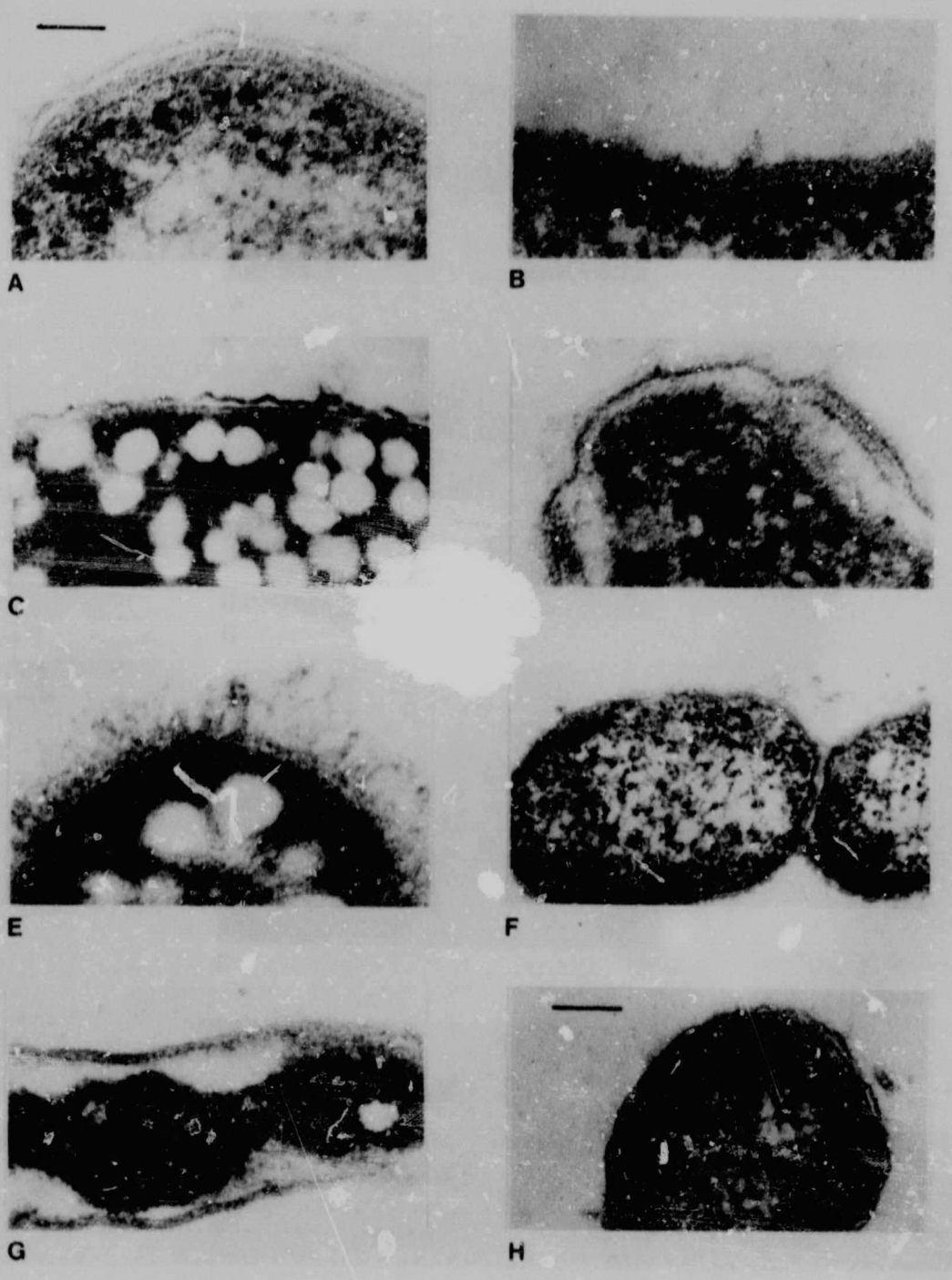

Figure 10. Classical Gram negative cell walls from TEM sections of cecal mucus. Bar = 0.1 µm

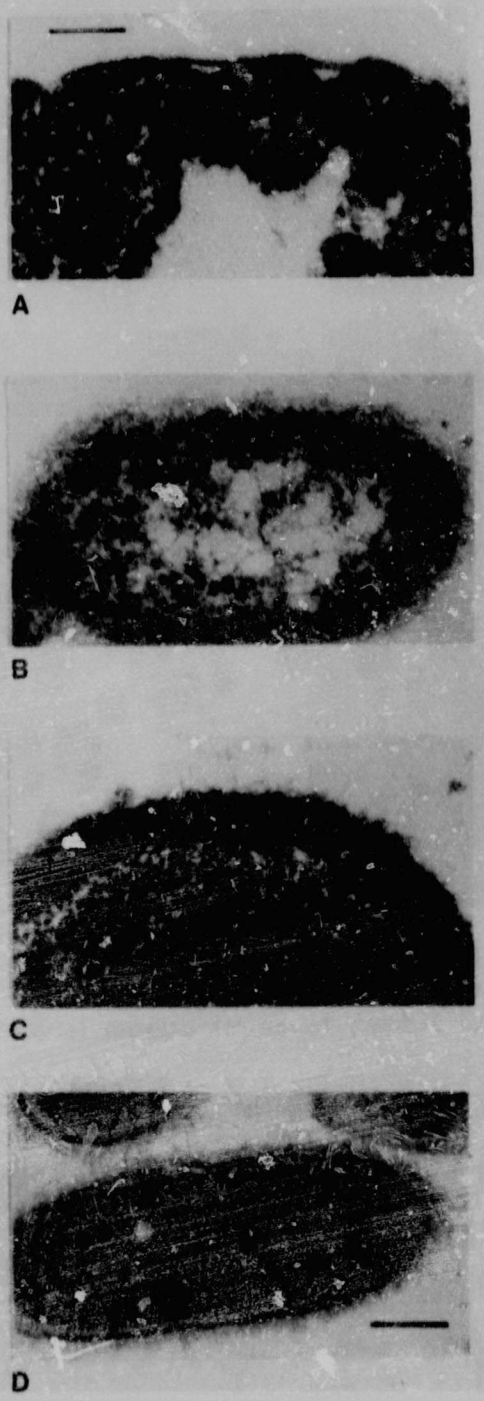

Figure 11. Cell walls from TEM sections of cecal mucus. This group did not conform to either of the classical structures. Bar = 0.1 μm

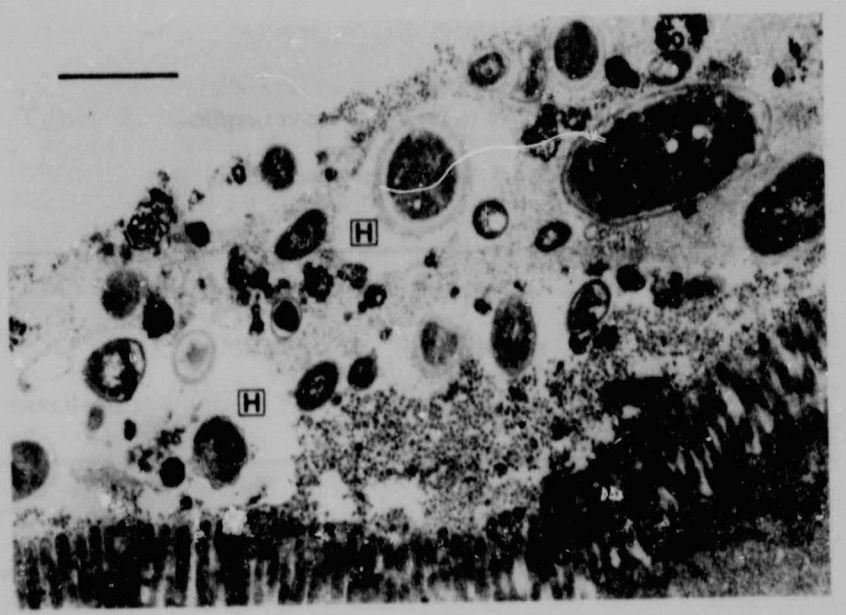

Figure 12. TEM of cecal mucus layer of rabbit cecum showing lucid halos,**H**, surrounding some bacteria. Bar = 1 µm

Table 2. Comparison of different media for primary isolation of cecal bacteria.

Culture medium	number of rabbits	Bacteria in digesta $x10^9$/ml	Bacteria in mucus $x10^6$/ml
Rumen fluid agar	n=3	4.4±2.1	5.1±0.9
Brain heart agar	n=3	3.1±0.8	2.3±2.0
Modified M10 agar	n=5	5.8±2.3	8.7±1.1

Data derived from duplicate plate count assays of dilutions of digesta and mucous scrapings from the ceca of rabbits. Counts are represented as the mean and standard deviation. Only the counts obtained with mucus on M10 medium are significantly different from counts on other media. $p=<0.05$.

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viable bacteria in scrapings from cecal mucosae was 10^6, in the range 10^5 to 10^8 per ml. The mean direct microscopic counts on suspensions of digesta and mucosal scrapings were 10^{10} and 10^8 per ml respectively.

3.3 Identification of isolates

Two hundred and twenty six isolates were initially studied, but 24 were lost before they were characterized. Approximately 40 were taken from each of 5 rabbits; 20 from lumen contents and 20 from mucous scrapings. Using 32 different characteristics, 59 isolates representative of the different sub-groups were presumptively identified. At least 95% correlation with published descriptions of the species was considered necessary before an isolate was assigned to a specific taxon. The strains were distributed among 19 different species as indicated in Table 3, and coded as W series from mucus scrapings and L series from the lumen contents. It should be re-emphasized at this point that identification of bacteria in this study was arrived at by closest-fit to published phenotypic patterns of known taxons. They are therefore, at the most, presumptive, as no attempt was made to compare genotypic characteristics with type strains. Nevertheless, the results serve to indicate the broad features of the bacterial flora inhabiting the rabbit cecum.

Members of the *Bacteroides fragilis*-like group were the most frequent isolates from both lumen and mucosa of all five rabbits. Until recently *Bacteroides vulgatus*, *Bacteroides ovatus* and *Bacteroides distasonis* were considered to be sub-species of *B. fragilis*, but are now considered to be separate species (Holdeman et al., 1984). They all have pleomorphic morphology in liquid media and similar colonial characteristics. Eight strains, W2, W12 W13, W22, L7, L34, L40, and L63 were identified as *Bacteroides vulgatus* together with 4 strains W6, L25, L36, L52 which did not ferment melibiose, although all grew well in the presence of bile. *Bacteroides vulgatus* has been recorded from the GIT and feces of man

Table 3. Results of tests used for presumptive identification of isolates cultured from the ceca of five rabbits.

Abbreviations.

N:Gram-negative. P:Gram-positive. R:rod. C:coccus. a:acid from substrate. w:weak reaction. +:positive for biochemical characteristic. -:negative for characteristic. T:terminal spore. ST:subterminal spore. Upper case for major metabolic-end products from glucose, lower case for minor products; A: acetic acid, P:propionic acid, B: butyric acid, ib: iso-butyric acid V: valeric acid, iv: iso-valeric acid, L: lactic acid, and S; succinic acid.

Note. All isolates gave negative reactions in the catalase test and in fermentation of inositol. These have not been included in the tables.

Table 3. Results of tests for the presumptive identification of representative isolates cultured from the rabbit cecum.

	Bacteroides vulgatus	*Bacteroides uniformis*	*Bacteroides ovatus*	*Bacteroides distasonis*	*Bacteroides ruminicola*	*Bacteroides oris*	*Bacteroides capillosus*
No. in group.	61	10	3	12	12	5	2
No identified.	12	3	1	5	3	2	1
Gram reaction.	N	N	N	N	N	N	N
Morphology	R	R	R	R	R	R	R
Arabinose	a	a	a	-	a	a	-
Cellobiose	a		a	w	a	a	-
Esculin hyd.	+	+	+		+	-	+
Fructose	a	a	a	a	a	a	w
Glucose	a	a	a	a	a	a	a
Galactose	a	a	a	a	a	a	a
Lactose	a	a	a	a	a	-	w
Maltose	a	a	a	a	a	a	-
Mannitol	-	-	a	a	-	a	-
Mannose	a	a	a	a	w	a	-
Melibiose	a/-	a/-	a	a	a	a	-
Raffinose	a	a	a	a	a	a	-
Rhamnose	a	a	a	-/w	-	a	w
Ribose	a	-	a	a	-	-/w	-
Salicin	-	a	a	a	a	w	-
Sorbitol	-	-	-	-	-	-	-
Starch hyd.	+	+	w	+	+	w	-
Sucrose	a	a	a	a	a	a	-
Trehalose	-	-	a	w/a	-	-	-
Xylose	a	a	a	a	-	a	w
Gelatin	+	-	+	+	+	-	-
Indole	-	+	+	-	-	-	-
Nitrate	-	-	-	-	-	-	-
VFAs	ASpiv	ASpib	ASp	ASpiv	ASb	ASl	AS
H_2S prod	+	+	+	-	-	-	-
Motility	-	-	-	-	-	-	-
20% Bile	+	w	+	+	-	-	-
Spore	-	-	-	-	-	-	-

Table 3. Continued.

	Bacteroides praeacutus	*Fusobacterium necrophorus*	*Fusobacterium mortiferum*	*Clostridium ramosum*	*Clostridium innocuum*	*Clostridium sticklandii*	*Eubacterium rectale*
No. in group.	1	4	9	3	5	2	7
No. identified.	1	1	5	1	2	1	3
Gram reaction.	N	N	N	N	P	P	P
Morphology.	R	R	R	R	R	R	R
Arabinose	-	-	-/w	-	-	-	a
Cellobiose	-	-	a	a	a	-	a
Esculin hyd.	-	-	+	w	+	-	+
Fructose	-	-	a	a	a	-	a
Glucose	-	-	a	a	a	a	a
Galactose	-	-	a	a	a	-	a
Lactose	-	-	a	a	-	-	a
Maltose	-	-	a/w	a	w	-	a
Mannitol	-	-	a	a	-	-	a/w
Mannose	-	-	a	a	a	-	a/w
Melibiose	-	-	a	a	-	-	a
Raffinose	-	-	a/-	a	a	-	a
Rhamnose	-	-	-	-	-	-	-/a
Ribose	-	-	w	a	a	a	-
Salicin	-	-	a	a	a	-	-
Sorbitol	-	-	-/w	a	-	-	-
Starch hyd.	-	-	-	-	-	-	+
Sucrose	-	-	a	a	a	-	a
Trehalose	-	-	a	w	-	-	-/a
Xylose	-	-	-	-	-	-	a
Gelatin	-	-	-	-	-	-	+/w
Indole	-	+	-	-	-	-	-
Nitrate	+	w	-	-	-	+	-
VFAs	Saf	ABp	Bapl	abf	abl	ABlib	BLas
H_2S prod	-	-	-	-	-	-	-
Motility	-	-	-	-	-	+	-
20% Bile	-	-	+	-	-	-	-
Spore	-	-	-	T	T	ST	-

Table 3. Continued.

	Ruminococcus albus	*Peptostreptococcus productus*	*Coprococcus eutactus*	*Leptotrichia buccalis*	*Streptococcus intermedius*	*Unclassified*
No. in group.	5	11	4	2	2	1
No. identified.	2	5	3	1	6	1
Gram reaction.	P	P	P	N	P	N
Morphology.	C	C	C	R	C	R
Arabinose	-	a	-	a	-	a
Cellobiose	a	a	a	w/-	a	-
Esculin hyd.	-	+	+	+	+	-
Fructose	-	a	a	a	a	a
Glucose	a	a	a	a	a	a
Galactose	-	a	a	a	a	a
Lactose	w	a	a/-	a	a	-
Maltose	-	a	a	a	a	-
Mannitol	-	a	-	-	-	-
Mannose	-	a	a	a	a	-
Melibiose	-	a	-	w/-	a	a
Raffinose	-	a	a	a	a	a
Rhamnose	-	a	-	-	-	a
Ribose	-	a	-	-	-	-
Salicin	-	-	a	-	-	-
Sorbitol	-	-	-	-	-	-
Starch hyd.	-/w	-/w	+	-/w	-	
Sucrose	-	a	a	a	a	a
Trehalose	-	a	-	a	-	-
Xylose	-	a	-	-	-	a
Gelatin	-	-	+	-	-	-
Indole	-	-	-	-	-	-
Nitrate	-	-	+	-	-	w
VFAs	A21	ALps	abip	I	Las	Apb
H_2S prod	-	-	-	-	-	-
Motility	-	-	-	-	-	-
20% Bile	-	-	-	-	-	-
Spore	-	-	-	-	-	-

and dogs (Macy & Probst, 1979), although it was not found in pig cecal contents (Robinson, et al.,1981).

Bacteroides ovatus (strains L32, L35, L82) is differentiated from *Bacteroides distasonis* (W29, W48, L17, L37, L73) mainly on indole production, but both are widely recorded from the intestinal tracts and feces of animals (Holdeman et al.,1984). Although the strains of *Bacteroides distasonis* from rabbit cecum characteristically grew well in bile, all of them also fermented mannitol. *Bacteroides uniformis* (W35, W53, L39) has also been found in pig ceca (Robinson et al., 1981; Russel,1979). One of the three strains identified from rabbit cecum (W53) failed to ferment melibiose, but was similar to W35 and W39 in all other respects. Two *Bacteroides* strains W2, and W56 were very similar to published descriptions of *Bacteroides oris* except for fermentation of mannitol. Although primarily known as part of the human oral flora, this species has been recorded from the chicken GIT and as the cause of peritoneal abcesses in man (Holdeman et al.,1984). A single isolate of a non-saccharolytic *Bacteroides* species (W78) gave a positive reaction only in the nitrate reduction test. As the organism was isolated from the cecum and had a pleomorphic morphology when grown in liquid culture, it was identified as *Bacteroides praeacutus* rather than the very similar *Bacteroides gracilis*, which has only been isolated from human gingival crevice. Another single isolate, W82, was similar to *Bacteroides capillosus* which has previously been isolated from pig ceca and the GIT of the chicken (Holdeman et al.,1977) **Volatile fatty acids from these strains may have come from other M10 components.** *Bacteroides ruminicola*, represented by isolates W51, L6, and L87, was found 4 out of 5 rabbits. They were not separated into sub-species on their haem requirements, although two of the strains could be placed in the biovar group 1 in the subspecies *brevis* of Bryant's taxonomic scheme, mainly on their inability to ferment xylose (Holdeman et al.,1984). *Bacteroides ruminicola* has been isolated from many GIT

habitats, notably the rumen, and as the most frequent *Bacteroides sp.* in the pig cecum and feces (Robinson et al., 1981; Robinson et al., 1984).

Two species of the genus *Fusobacterium* isolated from the rabbit cecum were characterized by their production of butyrate from fermentation of glucose. Strains W5, W85, W99, L19, and L80 showed most of the characteristics of *Fusobacterium mortiferum* and were found in 3 out of 5 ceca. Except for slight differences in the fermentation of a few sugars, the strains were all very similar to those isolated from human intestinal contents. (Holdeman et al., 1977). Two isolates (L18 & L80), conforming to the description of *Fusobacterium necrophorum*, fermented no sugars, and except for weak nitrate reduction, was similar to fusobacteria isolated from pig ceca, (Robinson et al.,1984) and to ureolytic strains identified in rabbit feces (Crociani et al., 1984).

Two similar isolates of a Gram-negative organism differing from *Leptotrichia buccalis* in fermentation of melibiose and trehalose, were broad, non-motile rods, producing only lactose from glucose fermentation. Despite its name, similar bacteria were identified in both lumen contents and mucosal scrapings from pig ceca (Robinson et al., 1984) and from human intestines (Holdeman et al.,1977).

Among the Gram-positive bacteria in the rabbit cecum, the facultative streptococci were by far, the most frequent species identified. They were recovered from both the lumen contents, and in lower numbers, from the mucosa of all animals. Six of the 21 isolates were found to be similar to *Streptococcus intermedius* (Holdeman et al., 1977), but judging from the many descriptions of *Streptococcus* species that have been isolated from pig, dog, human and rabbit GIT but identified only to genus level, the other 15 isolates in this study may well fall into a number of different taxa.

feces described by Crociani et al.(1984) and to urease-producing cocci in human feces (Wozny et al.,1977). Another genus was represented by five isolates two of which, W52 and L93 identified and found to be similar to *Ruminococcus albus* and it is assumed that the the other isolates also fell into this group. The taxon was found in low numbers associated with lumen contents from four of the five rabbits, although a single strain was isolated from mucus in rabbit number two. Primarily known as a cellulolytic member of the rumen flora (Hungate,1966), *R. albus* has been recovered from the GIT of many animals including man (Holdeman et al.,1977), dogs (Davis et al.,1977), guinea pigs (Dehority, 1977) and probably from the rabbit (Hall, 1952). The third genus of gram-positive cocci were all isolated from mucous scrapings (W79, W80, W97). They differed only in their reactions to starch and lactose and conformed closely to the descriptions of *Coprococcus* strains from the rumen (Wozny et. al.,1977) and from the pig cecum (strain A1 of Robinson et al., 1984). Specific names were not given in these reports, but the strains isolated from the rabbit cecum are sufficiently similar to the descriptions of *Coprococcus eutactus* given by Holdeman and Moore (1974) for presumptive identification.

Several isolates could not be placed in genera according the the identification keys of Holdeman et al.(1977) or Holdeman et al.(1984). Isolate L14 was a broad, pleomorphic short rod, producing acetic, lactic and formic acids from glucose fermentation. A slender, motile gram-negative rod (W34) did not survive freezing, and there were several thin sinuous streptobacilli producing acetic and different minor acids from glucose (L17, L66, W27). These minor populations were found very infrequently and were not investigated further in this study.

3.4 Distribution of bacterial species in the cecum

The Gram-positive spore-formers were also difficult to identify. Representative isolates taken for characterization, after separation on spore morphology, gave three different species; *Clostridium innocuum* conformed to the ureolytic clostridia isolated from rabbit soft feces (Crociani et al., 1984). *Clostridium ramosum* has previously been reported from human and pig GIT (Holdeman et al., 1977) and *Clostridium sticklandii*, the only motile isolates recorded in this study, has also been isolated from several GIT habitats. The remaining Gram-positive, anaerobic sporeformers were single isolates with different morphological characteristics. Individually they constituted only 0.5% of the total flora of the lumen contents and were considered to be minor populations that may not even have been indigenous. They are grouped together as unidentified clostridia in the tables.

Eubacteria were recovered from 4 of the 5 rabbits in low numbers. Of the 7 isolates identified to genus level, 3 were studied further. W17 and L62 fermented rhamnose but L90 did not, and none of the three fermented salicin or sorbitol. Nevertheless, there was still good correlation with the descriptions of *Eubacterium rectale* in view of the variable fermentation patterns recorded for this species by Holdeman et al. (1977). Members of the genus *Eubacterium* have been found in the GIT of most animals, but the species recorded seem to differ according to author and the animals being studied. Robinson et al.(1981), Robinson et al.(1984) and Russel (1979), all working on the microbiology of the pig cecum, recorded quite different species of *Eubacterium* at different times, in different pigs, but *Eubacterium rectale* was not recorded.

Three genera of Gram-positive obligately anaerobic cocci were identified among the isolates from rabbit ceca. *Peptostreptococcus productus* was found only in 3 out of 5 animals but from both lumen and mucus. The 5 strains identified, (W3, W68, W69, L50 and L53), all showed the same reactions. They were similar to the ureolytic isolates from rabbit soft