The bacteriology of the small intestinal mucosa of free-living reindeer

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Abstract: Bacteria in close associaton with the intestinal mucosa are thought to protect the mucosa from pathogenic microorganisms. The pH of the small intestinal mucosa and the viable populations of aerobic and anaerobic bacteria associated with the proximal and distal jejunal mucosa, were measured in four free-living reindeer in winter. The anaerobic bacterial populations were characterized. The median pH of the mucosa of the duodenum was 6.6 (n=4) at point 0.2 m from the pyloric sphincter. The mucosal pH increased along the length of the intestine to 8.3 at 14 m and then decreased to 7.9 at 19.8 m from the pyloric sphincter. Examination by transmission electron microscopy and cultivation techniques failed to reveal any bacteria on the mucosa of the proximal jejunum in two of the animals. In two other reindeer the median anaerobic bacterial densities in the proximal jejunum ranged from 25–2500 cells/g mucosa. The median anaerobic bacterial populations in the distal jejunum ranged from 80 to 20000 bacteria/g mucosa (n=4). The anaerobic population of bacteria in the proximal jejunum was dominated by streptococci and unidentified gram positive rods. Bacteroidaceae, streptococci and unidentified gram positive rods were common in the distal jejunum. The low density and the species diversity of bacteria in the small intestine suggests that these microorganisms are inhibited by components in the natural winter diet of reindeer. Bacteria evidently play a minor role in protection of the mucosa of reindeer in winter.

Key words: *Rangifer tarandus tarandus*, mucosa, bacteria, diet.

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Introduction

Studies of monogastric mammals have revealed the presence of indigenous microbial populations in the small intestine (Savage, 1977; Dubos & Schaedler, 1964). Bacteria associated with the mammalian intestinal mucosa are thought to protect the epithelium from pathogenic microorganisms (Shedlovsky & Freter, 1974; Jonsson, 1985). The population of adherent bacteria in the small intestine of sheep can be as high as 12000 cells/g tissue 2—3 m from the pylorus (Nickoletti *et al.*, 1984). Little is known about the microbiota of the small intestine of reindeer (*Rangifer tarandus).* Semi-domesticated reindeer in northern Norway range freely and eat lichens e.g. *Cladonia* spp., woody plants, grasses and sedges in winter (S. D. Mathiesen, unpubl.). Organic acids present in plant species such as *Betula nana* and *Vaccinium vitis-idaea* may reduce bacterial growth (Matzner, 1971; Radwan & Crouch, 1974; Bryant *et al.*, 1985). Usnic acid and other secondary metabolites in lichens are known to have antibacterial effect on soil bacteria (Vartia 1949, 1973). The mucosa of the jejunum (5—6 m from the pylorus) of captive reindeer fed a pure lichen diet *(C. stellaris)* is nevertheless known to be colonized by anaerobic bacterial populations as

high as 41500 cells/g, and dominated by lactic acid producing *Streptococcus* spp. and *Escherichia coli* (Sørmo & Mathiesen, 1993). The aim of the present study was to compliment our previous work by investigating the bacterial populations associated with the mucosa of the small intestine of free-living reindeer feeding on a natural winter diet.

Material and methods

Animals

Four adult (> 2 years), female reindeer (*R. t. tarandus*) grazing on a natural winter pasture near Guovdageaidnu, Norway (69°N, 23°E) were killed in winter (March 1991) by decerebration and bleeding in a traditional Same fashion. This was performed by skilled reindeer herders. A laboratory was established in the slaughterhouse at Guovdageaidnu. The animals were transported to the laboratory where measurements commenced within 20 min. of death. The body mass of each animal was measured using a Salter model 235 scale (100 ± 0.5 kg) immediately upon arrival.

pH

The gastrointestinal tract was removed within 30 min. of death and stripped of connective tissue and fat. The length of the small intestine containing digesta was measured to 1.0 cm after gentle stretching. The pH of the epithelial surface of the small intestine was measured at 2 m intervals beginning 20 cm from the pyloric sphincter. Measurements were taken within 35 min. of death using a calibrated, portable pH meter (PHM 80, Radiometer®, Copenhagen). The electrodes of the pH metre were placed directly on the mucosal surface. A 242C electrode and a reference electrode (K 401) was used.

Bacteria

Approximately 2 m long sections of the proximal jejunum (4.1 to 6.1 m from the pyloric sphincter) and of the distal jejunum (16.2—18.2 m from the sphincter) were ligated at both ends, cut out and transferred to an anaerobic glovebox (COY Laboratory products, Inc., Ann Arbor, Michigan, USA) maintained with an atmosphere in the box of 95% N_2, 5% H_2, at 39°C. The sections were emptied, cut into 10 cm segments which each of was opened by cutting longitudinally. The segments were washed vigorously in 500 ml anaerobic buffer (M8) and samples (5g wet weight) of the mucosa, including the epithelium, were scraped off randomly selected segments under sterile conditions with a scalpel. Each sample was homogenized in 45 ml M8 buffer containing 0.1% methylcellulose (Kudo *et al.*, 1987) in a Polytron PT 10 OD homogenizer (Kinematica, GMB, Luzern, Schweiz) for 30 sec. at a speed setting of 5.

Media

M8 anaerobic buffer (Hungate, 1950; Bryant & Robinson, 1961; Dehority & Grubb, 1976) formed the basis of the anaerobic habitat-simulating medium (M8V) (Orpin *et al.*, 1985b) used for the viable count determinations. M8 buffer (1 l, pH 6.8) was prepared by taking 150 ml mineral solution 1 and 150 ml mineral solution 2 (Bryant & Burkey, 1953), 10.0g tryptone, 2.5g yeast extract, 6.0g $NaHCO_3$ and 20% (v/v) sheep rumen fluid supernatant which had been prepared previously by filtering rumen fluid through two layers of muslin, incubating for 24 h at 39°C and centrifuging (27000 x g, 3.5 h). Resazurin (3.0 ml of a 0.1% (w/v) solution) was added as an oxygen indicator and 0.01% (w/v) L-cysteine HCl was included as a reducing agent. The solution was made up in bulk to a total of 1000 ml with distilled water. The habitat simulating medium (M8V) was made up in bulk and contained glucose, sucrose, maltose, cellobiose, starch, pectin and xylan, each at a concentration of 0.2% (w/v). It was solidified using 2.0% (w/v) agar, gassed in CO_2 and autoclaved at 115°C in 15 min. A solution of vitamins (10.0 ml per l medium) (Rochè *et al.*, 1973) was sterile filtered through a Millex® -GS single use filter unit (0.22 µm) (Millipore S. A., Molsheim, France) and added to the medium before use. To enumerate aerotolerant organisms, 8g nutrient broth (Difco) in 1 l distilled water was used. The broth which contained 0.2% (w/v) glucose as sole carbohydrate was solidified using 2.0% (w/v) agar and autoclaved for 20 min. at 115°C.

Enumeration of bacteria

The tissue suspensions from the proximal and distal jejunum were diluted serially in ten fold steps in M8 buffer. The population of bacteria present in dilutions of 10^{-1} to 10^{-4} of the homogenized mucosa were made viable by inoculating 1 ml from each dilution in quadruplicate in petri-dishes in the anaerobic chamber. Melted nutrient agar (Difco) or M8V medium cooled to 40°C was added to petri-dishes and mixed thoroughly with the inoculum. The petri-dishes containing nutrient agar were incubated aerobically. The petri-dishes containing M8V were put in an anaerobic jar in the anaerobic chamber and gassed with CO_2. All plates were incubated at 39°C for 48 h. Colonies of viable aerobic and anaerobic bacteria were then counted and results presented as number of bacteria per gram wet weight of mucosa.

Isolation and identification of bacteria

Bacterial colonies were picked from the anaerobically incubated plates using sterile tooth-picks (Orpin *et al.*, 1985a). Where bacteria were abundant, all colonies were picked from one quarter of each of the four petri-dishes from that dilution which had originally contained between 60 and 100 bacteria, until 50 colonies had been isolated. This process was repeated for samples from both the proximal and distal jejunum. Where there were few bacteria, all colonies present in the agar-plates from the 10^{-1} dilution were collected. The bacteria were streaked on M8V medium in new petri-dishes and were plated repeatedly until pure cultures were obtained. The bacteria were then transferred to individual Hungate-tubes containing an agar slope of M8V medium, incubated for 24 h and stored at -80°C until identification. The bacterial isolates were identified by examination of morphology and motility in liquid M8G (M8 buffer added 0.5% glucose), Gram staining after growth in liquid M8G medium for 4 h in 39°C, spore formation, substrate utilization pattern, and identification of acidic fermentation products (Holdeman & Moore, 1973; Krieg & Holt, 1984; Sneath *et al.*, 1986; Orpin *et al.*, 1985a; Ogimoto & Imai, 1981). The substrate utilization pattern was determined using solidified M8 buffer without carbohydrates containing 2.5% sheep rumen fluid and 2.0% agar. Seven different M8 media with 2.0% agar were prepared, each containing one of the following carbohydrates at a concentration of 0.5%: glucose, maltose, sucrose, cellobiose, starch, pectin and xylan (Orpin *et al.*, 1985a). The media were autoclaved for 15 min. at 115°C before use. The bacteria were replica plated anaerobically from a master plate containing eight different bacterial isolates inoculated on the M8V agar plate, on the plates with the different carbohydrates and on the control plate to which no carbohydrates had been added. Bacterial growth was determined after anaerobic incubation for 24—48 h at 39°C, with reference to the control plate (Orpin *et al.*, 1985b). Aerobic growth was tested on nutrient agar (Difco) containing 0.2% glucose.

Acidic fermentation products were determined after growth in liquid M8G medium for 2 x 24 h. Five ml of the bacterial suspension were fixed in 1.25 ml 0.5 M HCl. A subsample (1 ml) was withdrawn to which was added 0.5 ml concentrated HCl, 2 ml diethyl ether and 500 µg 2-ethyl butyric acid as internal standard. The ether phase was removed using a glass Pasteur pipette, before extraction once more with 1 ml ether. N-tert-buthyldimethylsilyl-N-methyltrifluoracetamide (100 µg) was added to the combined ether extract (1 ml) in a test tube, which was sealed and heated at 80°C for 20 min. The test tubes were kept at room temperature over night before injecting 0.5 µl to a chromatograph. The concentrations of volatile fatty acids, lactate and succinate was determined by gas liquid chromatography (Chrompack CP 9000) using a CP -SIL 8 CB column (Chrompack 7452, 30 m, 0.25 mm ID) containing a 0.25 µm film of silica gel. The bacteria were characterized by use of the Anaerobe laboratory manual (Holdeman & Moore, 1973), and by Bergey's manual of systematic bacteriology (Krieg & Holt, 1984; Sneath *et al.*, 1986).

Transmission electron microscopy (TEM)

Small sections (0.5 x 0.5 cm) of the proximal and distal jejunal wall were fixed in 4.0% glutar-aldehyde in 0.1 M Sørensens phosphate buffer (pH 7.0). TEM preparations of the

fixation-buffer, centrifuged to a pellet, were post fixed in 1.0% OsO_4 for 2 h, stained in uranyl acetate (5.0 %) for 1.5 h, and dehydrated in an ethanol series. Pellet blocks were embedded in Epon/Araldite resin and sectioned on an ultramicrotome. The sections were contrasted with uranyl acetate and Reynolds lead citrate and inspected by transmission electron microscopy (JEOL-JEM 1200 EX).

Statistics

Numerical results are expressed as median and range. Differences in bacterial population density between treatments were compared using the Wilcoxon rank-sum test (Bhattacharyya & Johnson, 1977). H_0 was rejected at $P<0.05$.

Results

Animals

The median total body mass of the four adult reindeer was 64.1 kg (range: 61.5—87.5 kg). The median length of the small intestines was 19.8 m (range: 19.5—22.0 m).

pH

The median pH of the mucosal surface increased along the length of the small intestine from the duodenum to a point in the jejunum 14 m from the pyloric sphincter. It then decreased to 7.66 at a point 18 m from the pyloric sphincter and increased again to 7.86 in the distal ileum (Table 1).

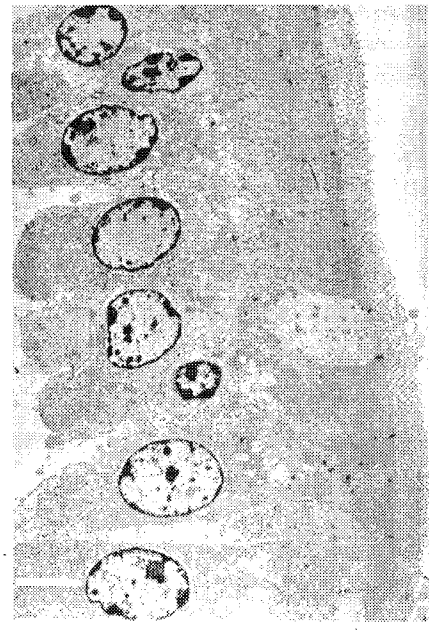

Fig. 1. Transmission electron micrograph showing microvilli on the mucosal surface of the proximal jejunum. No bacteria were observed on the villi. Bar = 5 μtn.

Table 1. The surface pH of the mucosa of the small intestinal epithelium of four free-living reindeer measured at different distances from the pyloric sphincter.

Distance from the pyloric sphincter (meter)	pH				
	1*	2*	3*	4*	median (range)
0.2	6.80	6.71	6.38	6.49	6.60 (6.38–6.80)
2	6.42	6.45	6.41	6.73	6.43 (6.41–6.73)
4	6.72	6.71	6.58	6.62	6.67 (6.58–6.72)
6	6.93	6.66	6.70	6.63	6.68 (6.63–6.93)
8	7.71	7.04	7.10	7.11	7.11 (7.04–7.71)
10	7.54	7.55	7.55	6.87	7.55 (6.87–7.55)
12	8.41	7.92	7.89	7.53	7.91 (7.53–8.41)
14	8.38	8.43	8.15	8.23	8.31 (8.15–8.43)
16	7.40	7.93	7.52	8.01	7.73 (7.40–8.01)
18	7.80	7.97	6.94	7.51	7.66 (6.94–7.97)
19.8	8.15	8.84	7.56	6.94	7.86 (6.94–8.84)
22	–	–	7.94	–	7.94**

*: reindeer No.
**: n=1.

Viable counts

No bacteria were detected on the proximal jejunal mucosa in two reindeer (Table 2) either by aerobic or anaerobic cultivation techniques or by TEM observations. Figure 1 shows a part of a villus in the proximal jejunum of one of these two reindeer (No. 3). The tissue is covered by a dense layer of microvilli and there is a complete absence of bacteria. The proximal jejunal mucosa of reindeer Nos. 1 and 2, by contrast, supported populations of aerobic bacteria of 20 and 1600 cells per gram tissue and of anaerobic bacteria of 25 and 2500 cells per gram tissue, respectively (Table 2). The population density of aerobic bacteria in the proximal jejunum was similar to the density of the anaerobic population (Table 2). Only 25.3% of 75 bacterial strains isolated from the anaerobic population in the two reindeer were facultatively anaerobic (Table 4 and 5). This indicates that the bacterial population associated with the proximal jejunal mucosa of reindeer Nos. 1 and 2 consisted of a mixture of strict anaerobic, facultative anaerobic and strict aerobic species.

All animals had bacteria on the mucosa of the distal jejunum (Table 2). The density of the aerobic population of bacteria in the distal jejunum of reindeer Nos. 1 and 3 was only 19.1 and 56.3%, respectively, of the population of anaerobic bacteria (Table 2). In reindeer Nos. 2 and 4, by contrast, the density of the aerobic populations of bacteria was higher than the density of the anaerobic population of bacteria. The densities of the anaerobic populations were 71.4 and 30.3% of the density of the aerobic populations in reindeer Nos. 2 and 4, respectively. A total of 141 bacterial strains were isolated anaerobically from the distal jejunum of the four reindeer. An average of 23.4% of the strains were facultatively anaerobic (Tables 6—9).

Identification of anaerobic bacteria

The morphological and chemical characteristics of bacteria isolated from the proximal and distal jejunal mucosa are presented in Tables 4—9. There were evidently substantial differences in species diversity of bacteria between animals (Table 3). The anaerobic bacterial population isolated from the proximal jejunal mucosa of reindeer No. 1 was dominated by *Streptococcus* spp. and an unidentified gram positive, strictly anaerobic rod (strain U 1) which produced mainly acetic acid and isocaproic acid when grown in liquid M8G (Table 4). Reindeer No. 2 had a more diverse bacterial population associated with the proximal jejunal mucosa, consisting of *Bacteroides* spp. (strain B6, B7, B10 and B13), *Fusobacterium* spp. (strain B4 and B5), *Streptococcus* spp. (strain S6—9) and *Clostridium* spp. (strain C1—4), *Selenomonas* sp. (strain B8), *Lactobacillus* sp. (strain L1) and *Propionibacterium* sp. (strain P1), (Table 5).

Streptococcus spp. and bacteria belonging to the family Bacteroidaceae were common in the distal jejunum in all animals (Table 3). In reindeer No. 1, *Butyrivibrio* sp. (strain B14), *Bacteroides* sp. (strain B15) and *Eubacterium* sp. (strain E1) were found in addition to *Bacillus* spp. (strain Ba1 and Ba2) and *Lactobacillus* sp. (strain L2), (Table 6).

Reindeer No. 2 had *Bacteroides* spp. (strains B16—20), and the unidentified strict anaerobic, gram positive rods (strains U11—12) in the distal jejunum (Table 7). In reindeer No. 3, *Selenomonas* sp. (strain B25), *Butyrivibrio* sp. (strain B27) were present in addition to the

Table 2. Number of viable aerobic and anaerobic bacteria [median (range)] per gram tissue (wet weight) of the small intestine in free-living reindeer grazing on a winter pasture.

Reindeer	Aerobic		Anaerobic	
	Proximal jejunum	Distal jejunum	Proximal jejunum	Distal jejunum
1	20 (20–70)	20 (20–30)	25 (20–30)	105 (90–120)
2	1600 (1500–2000)	7000 (6000–11000)	2500 (2400–2600)	5000 (3000–6000)
3	ND	45 (30–90)	ND	80 (70–130)
4	ND	66000 (63000–74000)	ND	20000–(20000–40000)

ND = Bacteria not detected.

Table 3. Proportion (%) of different types of bacteria isolated from the mucosa in the proximal and distal jejunum of free-living reindeer grazing on a natural winter pasture.

Reindeer number	1		2		3		4	
Site of isolation in jejunum (number of strains)	Proximal (18)	Distal (28)	Proximal (57)	Distal (46)	Proximal (0)	Distal (26)	Proximal (0)	Distal (41)
Bacterial groups[a]:								
Bacteroidaceae	16.7	17.9	68.4	76.1	-	38.5	-	61.0
Streptococcus spp.	50.0	21.4	15.8	6.5	-	11.5	-	2.4
Lactobacillus spp.	-	3.6	1.8	2.2	-	-	-	-
Clostridium spp.	-	-	12.3	-	-	-	-	-
Propionibacterium spp.	-	-	1.8	-	-	-	-	2.4
Eubacterium spp.	-	3.6	-	-	-	3.8	-	2.4
Bacillus spp.	-	7.1	-	-	-	-	-	-
Ruminococcus sp.	-	-	-	-	-	-	-	12.2
Others	33.3	46.4	-	15.2	-	46.2	-	19.6

[a] Differential characteristics of the bacterial groups are presented in Tables 4–9, see Appendix.
-: Bacterial groups not detected.

unidentified gram positive rod (strain U19) (Table 8). *Bacteroides* spp. (strains B28—29, and B31), *Ruminococcus* sp. (strain R1), *Propionibacterium* sp. (strain P2), *Eubacterium* sp. (strain E3) and unidentified gram positive, single cocci (strains U23—24) were found in reindeer No. 4 (Table 9). The bacterial strains U5, U11, U12 and U19 (Tables 5—8), were probably similar to strain U1 (Table 4).

Discussion

Two of the animals in this study appeared to have no bacteria associated with the mucosa of the proximal part of the jejunum. Similarly, in the remaining two reindeer, the population densities of aerobic and anaerobic bacteria at this site were low. In contrast to this, all four reindeer had populations of bacteria associated with the mucosa of the distal part of the jejunum (Table 2). There are several potential explanations for the difference in bacterial density between the proximal and distal parts. For example, motility decreases caudally along the jejunum and the ileum (Grovum & Williams, 1973), presumably resulting in better growth conditions for bacteria at increasing distance from the pyloric sphincter. In sheep, likewise, the population density of bacteria is known to increase along the small intestine (Nickoletti *et al.*, 1984; Hoogenraad & Hird, 1970).

Many of the bacterial strains which we isolated from the mucosa of the small intestine have previously been isolated from the rumen fluid of free-living reindeer killed in winter (T. H. Aagnes, unpubl.). For example, approximately one quarter (26.3%) of 80 bacterial strains isolated anaerobically from the rumen fluid of the free-living reindeer were facultatively anaerobic (T. H. Aagnes, unpubl.). In the present study 25.3 and 23.4%, respectively, of the small intestinal bacterial strains isolated anaerobically from the proximal and the distal jejunum were facultatively anaerobic. Likewise, bacteria belonging to the family Bacteroidaceae were common in the rumen fluid of free-living reindeer, (T. H. Aagnes, unpubl.) and these bacteria were also present at high population densities in the mucosa of the small intestine (Table 3). Streptococci, which were found at high population densities in the rumen fluid of two of four free-living reindeer (T. H. Aagnes, unpubl.) were also frequently occurring on the mucosa of the small intestine (Table 3).

Notwithstanding these observations, there were also distinct differences between the species composition of the microorganisms in

the rumen fluid and those associated with the mucosa of the small intestine. For example, only 3 of 80 isolates from a 10^{-8} dilution of rumen fluid of free-living reindeer (T. H. Aagnes, unpubl.) were similar to the unidentified strains of gram positive rods isolated from the mucosa of the small intestine. Here, the gram positive rods contributed 8.8% of strains of bacteria isolated. In addition, the *Bacillus* spp., *Lactobacillus* spp., *Selenomonas* spp. and *Eubacterium* spp. which we isolated from the small intestine have not been isolated from the rumen fluid of reindeer taken from the same herd from which our specimens came. These bacteria are commonly found in the rumen (Ogimoto & Imai, 1981), but probably exists at population densities lower than 10^8cells/ml rumen fluid which may explain why they were not detected. The strains of *Lactobacillus* spp. (L1—L3) and *Selenomonas* sp. (B8) which produced lactate as the major fermentation product (Tables 5—7) were more tolerant of low pH than most other rumen bacteria (Sneath *et al.*, 1986; Krieg & Holt, 1984). It is therefore possible that some of these bacteria, present at low population densities in the rumen, might survive the acidic environment in the abomasum and thus be able to establish themselves on the mucosa of the small intestine.

The fact that no bacteria were found on the mucosa of the proximal part of the jejunum in two of the reindeer might reflect individual differences in HCl secretion in the abomasum. The pH of the contents of the abomasum is known to be sufficiently low to destroy most of the microorganisms which enter from the omasum (Hungate, 1966), and we observed that the bacterial density in the proximal jejunum was significantly lower in free-living reindeer than in captive lichen-fed reindeer (W_S=11.5, n_1=4, n_2=5, P<0.05) (Sormo & Mathiesen, 1993). The crude protein content of plant material collected in winter from the rumen of animals from our study herd was about 10% (S. D. Mathiesen, unpubl.). This is substantially higher than in lichens, in which the crude protein may be less than 4.0% (Jacobsen & Skjenneberg, 1972). Protein and peptides in the abomasum enhance HCl secretion (Argenzio, 1984) and it is therefore possible that the abomasum of the free-living reindeer represents a significally more alien environment for bacteria than the abomasum of lichen-fed reindeer, owing to higher secretion of HCl. It is possible that individual differences in selection of diet influence HCl secretion which, in turn, resulted in sterile conditions on the mucosa of the proximal jejunum in two of the free-living reindeer.

Individual differences in the species composition and population densities of bacteria associated with the mucosa of the proximal and distal parts of the jejunum in our reindeer might also have been due to differences in flow rate of digesta through the small intestine. Rates of flow depend on the anatomy of the gastrointestinal tract and on the composition of the diet, both of which are individually variable. Flow rates also vary in different regions of the gut depending on the chemical and physical nature of the food, water consumption, salivary and intestinal secretions (Clarke, 1977).

A further explanation for the low bacterial densities associated with the proximal jejunal mucosa and for the individual differences in the species composition of the bacterial populations may be the influence of secondary metabolites in diet species. Organic acids and oils naturally occurring in *Vaccinium vitis-idaea* and *Betula nana* can inhibit growth and reduce the number of bacteria in the small intestine of the free-living animals (Radwan & Crouch, 1974). Moreover, usnic acid and other weak organic acids present in lichens are known to have anti-bacterial effects on soil bacteria (Vartia, 1949, 1973). It is not known what effect these acids have on the bacterial population of the small intestine in reindeer. We assume that they could be activated as bactericides in the abomasum, owing to the low pH there, and therefore inhibit bacterial growth in the small intestine while not in the rumen.

In conclusion, we have shown that free-living reindeer have low bacterial densities or even sterile conditions on the mucosa of the proximal part of the jejunum, and that the bacterial composition on the mucosa of the jejunum is highly variable. Consequently, it seems unlikely that maintenance of a fully functional mucosa in the small intestine requires an indigenous bacterial population in

reindeer as has been proposed for other species of mammals.

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Appendix: Tables 4–9

Table 4. Morphological and biochemical characteristics of bacteria isolated from the mucosa of the proximal jejunum from reindeer No. 1.

	Streptococcus spp.					Bacteroidaceae			Unidentified strains	
Strain: (number of strains)	S1 (4)	S2 (2)	S3 (1)	S4 (1)	S5 (1)	B1 (1)	B2 (1)	B3 (1)	U1 (5)	U2 (1)
Characteristics*										
Cell morphology	Cocci in chains	Cocci in chains	Cocci in chains	Cocci in chains	Small cocci in clusters	Short rod in chains	Short rod in chains	Slim rod in chains	Large rod, single or pairs	Irregular rod
Gram stain	+	+	+	+	+	–	–	–	+	+
Spores	–	–	–	–	–	–	–	–	–	–
Motility	–	–	–	–	–	–	–	+	+	+
Facultative anaerobic	+	+	+	+	+	–	–	–	–	–
Strict anaerobes	–	–	–	–	–	+	+	+	+	+
Utilization of:										
Glucose	+	+	+	+	+/–	+	+/–	+	+	+
Maltose	+	+	+	+	+/–	–	–	+/–	+	+
Sucrose	+	+	+	+	+/–	–	–	+/–	+	+
Pectin	–	–	–	–	–	–	–	–	–	–
Xylan	–	–	–	–	–	–	–	–	d	–
Cellobiose	+	+	+	+	+/–	+	+	+	+	+
Starch	+	+	+	+	+/–	+	+	+	+	+
Fermentation products**	L, a (p, b)	L, a, (p, b, ib, iv, v)	L, (a)	L, (b, a, ic)	c, (a, p, h, l, ic)	A, ic, l, ib, b, iv, (v)	l,b,a	L, a	A, ic/(ic), iv/(iv), b/(b), (l)	b, l, a

* Symbols: +, positive response; –, negative or no response; d, variable response; +/–, weak growth.

** Concentration of volatile fatty acids produced; a, acetate; p, propionate; b, butyrate; c, caproate; v, valerate; h, heptanate; l, lactate; s, succinate; iv, isovalerate; ib, isobutyrate; ic, isocaproate. Bold capital letters represent an amount of products equal to or greater than 1500 μg/ml, capital letters represent an amount of products from 500–1500 μg/ml, small letters represent 100–500 μg/ml and products in parentheses are produced in amounts less than 100 μg/ml.

Table 5. Morphological and biochemical characteristics of bacteria isolated from the mucosa of the proximal jejunum from reindeer No. 2.

	Streptococcus spp.				Bacteroidaceae										*Clostridium* spp.				*Lacto-bacillus* sp.	*Propioni-bacterium* sp.
Strain:	S6	S7	S8	S9	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	C1	C2	C3	C4	L1	P1
(number of strains)	(5)	(1)	(2)	(1)	(12)	(5)	(7)	(6)	(1)	(3)	(2)	(1)	(1)	(1)	(2)	(3)	(1)	(1)	(1)	(1)
Characteristics*																				
Cell morphology	Cocci in chains	Cocci in chains	Cocci in chains	Cocci in chains	Curved rod in chains	Curved rod in chains	Curved rod in chains	Short rod in chains	Curved rod in pairs	Curved rod in pairs	Short rod in chains	Short rod in chains	Short rod in chains	Irregular rod	Rod in chains	Rod in chains	Rod in chains	Rod in chains	Irregular rod	Irregular rod
Gram stain	+	+	+	+	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+	+
Spores	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	ND	+	+	–	–
Motility	–	–	–	–	–	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–
Facultative anaerobic	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Strict anaerobes	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Utilization of:																				
Glucose	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pectin	d	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+	+
Xylan	d	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	+
Cellobiose	+	+	+	+	d	d	–	–	–	–	–	–	–	+/–	+	+	+	+	+	+
Starch	+	+	+	+	+	+	–	–	–	–	–	–	–	–	+	+	+	+	+	+
Fermentation products**	L	L, (p, b, s)	L, (a, s, p)	L, a (p)	B, l	B, (l,a)	a, (l,s),	a, l/(l), (s)	L, (p, a)	A, ic, (iv, ib, s, p, c)	A, l, s, (p, b, v, iv, ib)	l, (s, b)	L, b, (s)	(a, l)	A, ic, (iv, ib)	b, l, a	b, l	ic, (iv, p, s)	L, a, (b, p)	p, a

* Symbols: +, positive response; –, negative or no response; d, variable response; +/–, weak growth; ND, not determined.

** Concentration of volatile fatty acids produced: a, acetate; p, propionate; b, butyrate; c, caproate; v, valerate; l, lactate; s, succinate; iv, isovalerate; ib, isobutyrate; ic, isocaproate. Bold capital letters represent an amount of products equal to or greater than 1500 μg/ml, capital letters represent an amount of products from 500–1500 μg/ml, small letters represent 100–500 μg/ml and products in parentheses are produced in amounts less than 100 μg/ml.

Table 6. Morphological and biochemical characteristics of bacteria isolated from the mucosa of the distal jejunum from reindeer No. 1.

	Streptococcus spp.			Bacteroidaceae		*Bacillus* spp.		*Lacto-bacillus* sp.	*Eubac-terium* sp.	Unidentified strains							
Strain:	S10	S11	S12	B14	B15	Ba1	Ba2	L2	E1	U3	U4	U5	U6	U7	U8	U9	U10
(number of strains)	(3)	(2)	(1)	(2)	(3)	(1)	(1)	(1)	(3)	(1)	(1)	(1)	(1)	(2)	(1)	(2)	(2)
Characteristics*																	
Cell morphology	Cocci in chains	Cocci in chains	Cocci in clusters	Slim, short rod	Irregular rod	Large rod in pairs	Large rod in pairs	Cocco-bacilli	Rod in pairs	Rod in pairs	Rod in pairs	Rod in pairs	Rod in pairs	Short rod	Short rod	Short rod in pairs	Short rod in pairs
Gram stain	+	+	+	–	–	+	+	+	+	+	+	+	–	–	–	–	–
Spores	–	–	–	–	–	+	+	–	ND	–	–	–	ND	–	–	–	–
Motility	–	–	–	+	–	–	+	–	+	+	–	+	–	+	+	+	–
Facultative anaerobic	+	+	+	–	–	+	+	+	–	–	–	–	–	+	+	+	+
Strict anaerobes	–	–	–	+	+	–	–	–	+	+	+	+	+	–	–	–	–
Utilization of:																	
Glucose	+	+	+/–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+/–	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+/–	+	–	+	+	+	+	+	ND	+	+	+	+	+	+	+
Pectin	–	–	–	–	–	–	+	–	+	ND	+	+	–	–	–	–	–
Xylan	–	–	–	–	–	–	+	–	+	ND	+	+	–	–	–	–	–
Cellobiose	+	+	+	+/–	+	+	+	+	+	ND	+	+	+	+	+	+	+
Starch	+	+	+	+/–	+	+	+	+	+	ND	+	+	+	+	+	+	+
Fermentation products**	L, a, (p, s)	L	L, a, ic, b, (ib, iv)	B, l, (a)	P, a, (iv, ib, h)	A, iv, ic, ib, (l)	L, (a, b)	L, a (p, s)	B, l, a	L, a, ic, (iv, ib, p)	A, (l)	A, ic, (iv, ib, s, l)	b, a, (l)	b, l, a	L	A, l	A, (s)

* Symbols: +, positive response; –, negative or no response; d, variable response; +/–, weak growth; ND, not determined.

** Concentration of volatile fatty acids produced: a, acetate; p, propionate; b, butyrate; c, caproate; v, valerate; h, heptanate; l, lactate; s, succinate; iv, isovalerate; ib, isobutyrate; ic, isocaproate. Bold capital letters represent an amount of products equal to or greater than 1500 μg/ml, capital letters represent an amount of products from 500–1500 μg/ml, small letters represent 100–500 μg/ml and products in parentheses are produced in amounts less than 100 μg/ml.

Table 7. Morphological and biochemical characteristics of bacteria isolated from the mucosa of the distal jejunum from reindeer No. 2.

	Streptococcus spp.			Bacteroidaceae							*Lactobacillus* sp.	Unidentified strains			
Strain: (number of strains)	S13 (1)	S14 (1)	S15 (1)	B16 (5)	B17 (12)	B18 (3)	B19 (6)	B20 (2)	B21 (6)	B22 (1)	L3 (1)	U11 (4)	U12 (1)	U13 (1)	U14 (1)
Characteristics*															
Cell morphology	Cocci in chains	Cocci in chains	Small cocci in tetraeder	Irregular rod in chains	Irregular rod in chains	Irregular rod in chains	Irregular rod in chains	Irregular rod in chains	Slim, curved rod	Slim, curved rod	Short rod	Large rod	Large rod	Rod	Rod
Gram stain	+	+	+	–	–	–	–	–	–	–	+	+	+	+	+
Spores	–	–	–	–	–	–	–	–	–	–	–	ND	–	–	–
Motility	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+
Facultative anaerobic	+	+	+	–	–	–	–	–	–	–	+	–	–	+	+
Strict anaerobes	–	–	–	+	+	+	+	+	+	+	–	+	+	–	–
Utilization of:															
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	–	–	+/–	–	–	+	–	+	+	+	+	+
Pectin	–	+	–	–	–	+/–	–	–	d	–	+	+	+	+	+
Xylan	+	+	–	–	–	–	–	+	d	–	+	+	–	+	+
Gellobiose	+	+	+	–	–	+	–	–	+	+	+	+	+	+	+
Starch	+	+	+	–	–	+	d	+	+	+	+	+	+	+	+
Fermentation products**	L, (a)	L, a, ic, (iv, ib, p)	L	a, p, (b,l)	a, p, (b)	a, p, (b)	a, p, (b, s)	a, p, (s)	B, (l)	B, a, (l, p)	L, a	A, ic, (ib, iv, v, c)	A, ic, iv, ib, (p, s)	A, (l, b)	A, l, (p, b s)

* Symbols: +, positive response; –, negative or no response; d, variable response; +/–, weak growth; ND, not determined.

** Concentration of volatile fatty acids produced: a, acetate; p, propionate; b, butyrate; c, caproate; v, valerate; l, lactate; s, succinate; iv, isovalerate; ib, isobutyrate; ic, isocaproate. Bold capital letters represent an amount of products equal ro or greater than 1500 μg/ml, capital letters represent aa amount of products from 500–1500 μg/ml, small letters represent 100–500 μg/ml and products in parentheses are produced in amounts less than 100 μg/ml.

Table 8. Morphological and biochemical characteristics of bacteria isolated from the mucosa of the distal jejunum from reindeer No. 3.

	Streptococcus sp.	Bacteroidaceae					*Eubacterium* sp.	Unidentified strains				
Strain:	S16	B23	B24	B25	B26	B27	E2	U15	U16	U17	U18	U19
(number of strains)	(3)	(6)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(8)
Characteristics*												
Cell morphology	Cocci in chains	Rods appear to be forked	Rods appear to be forked	Large, curved rod	Small, curved rod	Slim rod	Rod	Rod	Rod	Rod	Large rod	Large rod
Gram stain	+	–	–	–	–	–	+	–	+	–	–	+
Spores	–	–	–	–	–	–	–	–	–	–	–	–
Motility	–	–	–	+	–	–	–	+	+	+	–	–
Facultative anaerobic	+	–	–	–	–	–	+	–	–	+	ND	–
Strict anaerobes	–	+	+	+	+	+	–	+	+	–	ND	+
Utilization of:												
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+
Pectin	–	–	–	–	–	–	–	–	–	–	–	–
Xylan	–	–	–	–	–	–	–	–	–	–	–	–
Cellobiose	+	+	+	–	+	+	–	+	+	+	+	+
Starch	+	+	+	–	+	+	–	+	+	+	+	+
Fermentation products**	L	l, a, (s)	A, ic, (ib, b, v, s)	P, a, (l, s)	a, l	b, (l)	B, (l, s)	L	A	B, ib, l	a, (s)	A, ic, (ib, iv)

* Symbols: +, positive response; –, negative or no response; ND, not determined.

** Concentration of volatile fatty acids produced: a, acetate; p, propionate; b, butyrate; c, caproate; v, valerate; l, lactate; s, succinate; iv, isovalerate; ib, isobutyrate; ic, isocaproate. Bold capital letters represent an amount of products equal to or greater than 1500 μg/ml, capital letters represent an amount of products from 500–1500 μg/ml, small letters represent 100–500 μg/ml and products in parentheses are produced in amounts less than 100 μg/ml.

Table 9. Morphological and biochemical characteristics of bacteria isolated from the mucosa of the distal jejunum from reindeer No. 4.

	Streptococcus sp.	*Rumino-coccus* sp.	Bacteroidaceae					*Eubacterium* sp.	*Propioni-bacterium* sp.	Unidentified strains				
Strain:	S17	R1	B28	B29	B30	B31	B32	E3	P2	U20	U21	U22	U23	U24
(number of strains)	(1)	(5)	(11)	(10)	(1)	(2)	(1)	(1)	(1)	(1)	(1)	(1)	(4)	(1)
Characteristics*														
Cell morphology	Cocci pairs	Cocci, single or in pairs	Irregular rod	Irregular rod	Irregular rod	Rod	Slim, curved rod	Large rod	Rod appear to be forked	Curved rod in chains	Curved rod in chains	Small rod	Small, single cocci	Small, single cocci
Gram stain	+	+	–	–	–	–	–	+	+	+	–	+	+	+
Spores	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Motility	–	–	–	–	–	–	+	+	–	–	–	–	–	–
Facultative anaerobic	+	–	–	–	–	–	–	–	–	–	+	–	+	–
Strict anaerobes	–	+	+	+	+	+	+	+	+	+	–	+	–	+
Utilization of:														
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	–	–	+	–	–	–	–	+	–	+	–	–	–	–
Pectin	–	–	–	–	–	–	–	+	–	+	–	–	–	–
Xylan	–	–	–	–	–	–	–	+	–	+	–	–	–	–
Gellobiose	+	+	+	–	–	+	+	+	+	+	–	–	–	–
Starch	+	+	+	+	+	+	+	+	+	+	+	–	+	+
Fermentation products**	L, (b)	a, p, (l, s)	(a, p, s)	a, p, (s, l)	(s)	a, p, (s, l, b)	p, (s, l)	l, b, a, (s)	p, a, (s, l)	a, p, (b, s)	a, p, s, (l)	a, p, s, (l, iv)	a, (p, s, l)	B, L, p, a, (s)

* Symbols: +, positive response; –, negative or no response.

** Concentration of volatile fatty acids produced: a, acetate; p, propionate; b, butyrate; c, caproate; v, valerate; l, lactate; s, succinate; iv, isovalerate; ib, isobutyrate; ic, isocaproate. Bold capital letters represent an amount of products equal to or greater than 1500 μg/ml, capital letters represent an amount of products from 500–1500 μg/ml, small letters represent 100–500 μg/ml and products in parentheses are produced in amounts less than 100 μg/ml.