APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1994, p. 4445–4455
0099-2240/94/$04.00+0

Vol. 60, No. 12

Digestion of Herring by Indigenous Bacteria in the Minke Whale Forestomach

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Received 28 February 1994/Accepted 23 September 1994

Northeastern Atlantic minke whales (*Balaenoptera acutorostrata*) have a multichambered stomach system which includes a nonglandular forestomach resembling that of ruminants. Bacteria from the forestomachs of herring-eating whales were enumerated and isolated in an anaerobic rumen-like culture medium (M8W medium). The total viable population of anaerobic bacteria ranged from 73×10^7 to 145×10^8/ml of forestomach fluid ($n = 4$). *Lactobacillus* spp. (19.7%), *Streptococcus* spp. (35.9%), and *Ruminococcus* spp. (12.8%) were the most common of the bacterial strains ($n = 117$) isolated by use of M8W medium from the forestomach fluid population of two minke whales. Most of the isolates stained gram positive (93.2%), 62.4% were cocci, and all strains were strictly anaerobic. The population of lipolytic bacteria in one animal, enumerated by use of a selective lipid medium, constituted 89.7% of the viable population. The total viable population of anaerobic bacteria in freshly caught and homogenized herring (*Clupea harengus*) ranged from 56.7 to 95.0 cells per gram of homogenized prey ($n = 3$) when M8W medium was used. *Pediococcus* spp. (30.6%) and *Aerococcus* spp. (25.0%) were most common of the bacterial strains ($n = 72$) isolated from the homogenized herring. Most of the bacterial strains were gram positive (80.6%), and 70.8% were cocci. Unlike the forestomach bacterial population, as many as 61.1% of the strains from the herring were facultatively anaerobic. All bacterial strains isolated from the prey had phenotypic patterns different from those of strains isolated from the dominant bacterial population in the forestomach, indicating that the forestomach microbiota is indigenous. Scanning electron microscopic examinations revealed large numbers of bacteria, surrounded by a glycocalyx, attached to partly digested food particles in the forestomach. These data support the hypothesis that symbiotic microbial digestion occurs in the forestomach and that the bacteria are indigenous to minke whales.

Baleen whales, such as the small minke whales (*Balaenoptera acutorostrata*), which seldom exceed 9 m in length, have stomach systems consisting of four distinct compartments, including a forestomach resembling that of ruminants (28). The epithelial microanatomy of the forestomach is analogous to that of the rumen, but the epithelium lacks papillary projections to aid absorption of nutrients (28). High concentrations of volatile fatty acids (VFAs) and anaerobic bacteria in large baleen whales (12, 13), indicating forestomach microbial fermentation, also encourage functional comparisons with ruminants.

Northeastern Atlantic minke whales are known to feed on fish such as herring, capelin, and cod, in addition to pelagic crustaceans (24). These prey items may be degraded by bacterial fermentation to produce VFAs and bacterial protein. In minke whales the intestines are short, only four times the body length, and a multichambered stomach system improves the utilization of food (28). The development of a compartmentalized stomach system, in which retention of the food in a nonglandular forestomach allows growth of large numbers of anaerobic bacteria, makes microbial fermentation a tenable hypothesis. However, the functional importance of the forestomach bacteria in digestive processes is currently unknown.

The intention of this study was to examine the forestomach bacterial population of herring-eating minke whales. It remained to be clarified whether the bacteria are indigenous to the forestomach. In order to determine the origin of the forestomach bacterial population, representative bacterial strains isolated from the forestomachs of herring-eating minke whales had to be characterized and compared with those isolated from the prey. Substrates available for fermentation and the chemical factors that influence and select for different bacteria in the forestomach fluid were investigated. Scanning electron microscopy (SEM) of food particles from the forestomach was conducted to determine whether the bacteria contribute to digestion by attacking the prey in the forestomach.

MATERIALS AND METHODS

Animals and sampling. Samples were collected from four minke whales during Norwegian scientific whaling expeditions in August 1988 and August 1990 under licenses issued by the Norwegian government, in accordance with Article VIII of the International Convention for the Regulation of Whaling (1). The whales were caught along the coast of northern Norway, from the Vesterålen area (68.6°N) to West Finnmark (70°N), by small whaling vessels. General characteristics of each animal are presented in Table 1. Body length was measured in a straight line middorsally from top of the head to end of the fluke. Prey items found in the forestomach were identified according to standard external criteria or by otoliths (10). A standard microbiological laboratory was established aboard the whaling boat. Gastrointestinal tracts were removed immediately after the animals were killed, allowing incisions through the forestomach wall for sampling of contents within 30 min of death.

Forestomach pH. The forestomach contents of each animal were mixed in situ, and a subsample was filtered through two layers of muslin. The pH values of the subsamples were measured as described by Olsen et al. (28).

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TABLE 1. General characteristics of the forestomach contents and bacterial populations in the forestomachs of herring-eating minke whales

Whale no.	Characteristics of whale		Characteristics of forestomach contents										Viable bacterial cells (10^9/ml, mean ± SD)
	Sex[a,b]	Length (m)	pH	Vol (liters)[a]	% of vol as DM	% of total DM					Na (g/kg of DM)	Cl (g/kg of DM)	
						Ash	Crude protein	NH_4-N	Lipids	Water-soluble carbohydrates			
8-88	F	5.50[c]	6.32[c]	5	—[d]	—	—	—	—	—	—	—	14.5 ± 0.5
1-90	M	7.80	6.36	85	42.2	6.40	39.6	1.21	54.4	2.46	1.66	1.14	1.4 ± 0.2
2-90	M	8.00	5.36	61	38.4	4.95	39.4	0.44	58.5	1.74	2.34	1.46	0.73 ± 0.13
3-90	M	8.40	6.87	54	44.4	7.21	39.7	0.95	57.1	2.14	1.58	0.20	5.93 ± 1.05

[a] Data from reference 24.
[b] F, female; M, male.
[c] Data from reference 28.
[d] —, not determined.

Chemical and mineral analysis. Forestomach contents for chemical and mineral analysis were collected from minke whales 1-90, 2-90, and 3-90 and frozen immediately at −20°C. The forestomach contents were homogenized with a table homogenizer prior to analysis. The percentage and mass of dry matter (DM) were determined after the forestomach contents were preheated at 80°C for 24 h and then heated at 103 to 105°C for 4 h. For analyses of ash and mineral composition, forestomach contents were ashed at 550°C for 12 h. The ash was dissolved in aqua regia and evaporated until dry. The residue was then dissolved twice in 12.5 M HCl and evaporated until dry. Finally, after being dissolved again in HCl, the residue was diluted and filtered (15). Sodium content was determined by flame photometry (model 410 photometer; Corning, Halstead, Essex, England), and chloride content was determined by Volhard's method (15). Nitrogen content was determined by the Kjeldahl method (15) and converted to crude protein by multiplication by 6.25. Ammonia and other volatile nitrogen compounds (NH_4-N) were liberated from an aqueous extract of the forestomach contents by addition of magnesium oxide to give a pH of >7.5 when boiled, the distillate was allowed to react with H_2SO_4, and excess acid was titrated with NaOH solution (15). To evaluate the lipid contents, the forestomach contents were mixed with dried sodium sulfate to bind water, and the fat was extracted by ethyl acetate by the method of Losnegard et al. (20a). To determine the concentration of water-soluble carbohydrates, forestomach contents were extracted with water, filtered, hydrolyzed with H_2SO_4 in a water bath, neutralized with NaOH (34), and deproteinized with zinc sulfide-barium hydroxide, and the carbohydrate content was assayed by the ferricyanide method (8).

Enumeration of bacteria in forestomach fluid. All of the forestomach contents were removed from each animal, and a subsample (1.5 to 2.0 liters) was mixed thoroughly by hand and filtered through two layers of muslin for microbiological analyses. Colony counts of viable cells present in dilutions of forestomach fluid of 10^{-7} to 10^{-10} were done by the method of Hungate (16). The counts were done with Hungate anaerobic culture tubes (catalog no. 2047/16-125; Bellco, Vineland, N.J.), each fitted with a screw cap and a butyl rubber septum and containing an anaerobic rumen-like culture medium developed for comparative studies of whale bacteria (M8W medium). The techniques used for preparation of M8W medium were modified from those of Hungate (16) and Bryant and Robinson (3). M8W medium consisted of a basal medium (M8 medium) supplemented with 0.2% (wt/vol) glucose, *N*-acetyl-D-glucosamine (NAG), glycogen, maltose, cellobiose, and laminarin and solidified with 2% (wt/vol) agar. M8 medium contained 15% (vol/vol) mineral solution no. 1 and 15% (vol/vol) mineral solution no. 2 (both previously described by Bryant and Burkey [2]), 0.25% (wt/vol) yeast extract, 1% (wt/vol) tryptone, and 0.6% (wt/vol) $NaHCO_3$. M8 medium was prepared with 20% (vol/vol) preincubated sheep rumen fluid by a modification of the method of Dehority and Grubb (6). The sheep rumen fluid was strained through two layers of muslin, incubated for 24 h at 39°C, and centrifuged (27,000 × *g* for 3.5 h) before the supernatant fluid was removed for addition to the medium. Resazurin (3.0 ml of a 0.1% [wt/vol] solution) was added as an oxygen indicator, and 0.01% (wt/vol) L-cysteine HCl was added as a reducing agent. The solidified M8 medium together with the carbohydrates was made up in bulk to a total of 1,000 ml with distilled water, gassed with CO_2 to a final pH of 6.8, and dispensed with CO_2 into the Hungate tubes. The tubes were then sealed and autoclaved at 115°C for 15 min. Vitamins (0.1 ml per 10 ml of medium) previously described by Roché et al. (31) were sterile filtered into the medium before incubations. Dilutions of the forestomach fluid were made in liquid M8 medium without carbohydrates; inoculated in quadruplicate into Hungate tubes containing M8W medium for each animal; and incubated at 35°C, the temperature recorded in the forestomach, for 48 h aboard the whaling boat. The bacterial colonies in each Hungate tube were counted, and the numbers are expressed as the mean ± standard deviation from the mean of the population of bacteria per milliliter of forestomach content. In one animal (no. 8-88), the lipolytic bacterial population was determined with a selective lipid M8 medium lacking sheep rumen fluid and including 0.2% (wt/vol) triolein (catalog no. T 7140; Sigma) and 2% agar. For the same animal, numbers of bacteria using NAG were determined by growth on M8 medium with 0.5% (wt/vol) NAG as the sole carbohydrate. Both the lipolytic medium and the NAG medium were prepared in Hungate tubes.

Enumeration of bacteria in herring. Freshly caught herring (*Clupea harengus*) were homogenized in an autoclaved table homogenizer and immediately transferred to an anaerobic glove box (Coy Laboratory Products, Inc., Ann Arbor, Mich.) with an atmosphere of N_2, CO_2, and H_2; a palladium catalyst present to remove O_2; and a temperature of 35°C. The homogenized herring (5 g) was diluted in 45 ml of M8 medium in a 100-ml sealed bottle, and subsequent dilutions were made in liquid M8 medium in Hungate tubes. Colony counts of viable cells present in 10^{-1} to 10^{-5} dilutions of homogenized herring (n = 3) were done with M8W medium made up in 25-ml quantities in petri dishes under a CO_2 atmosphere inside the anaerobic glove box. The petri dishes were inoculated in

quadruplicate from each dilution and incubated for 48 h in sealed buckets gassed with CO_2 inside the anaerobic chamber. The bacterial colonies in each petri dish were counted, and the numbers are expressed as means ± standard deviations from the mean of the population of bacteria per gram (wet weight) of freshly caught and homogenized herring.

Isolation and identification of bacteria. Bacterial cultures from the forestomach fluid of two minke whales (no. 2-90 and 3-90) in Hungate tubes containing M8W medium were isolated and identified in our laboratory in Tromsø, Norway. Bacterial colonies were picked randomly with sterile glass Pasteur pipettes, from 10^{-7} and 10^{-8} dilutions in culture tubes, and from petri dishes inoculated with 10^{-1} dilutions of bacteria from freshly caught and homogenized herring ($n = 3$). All of the microbial work was performed inside the anaerobic chamber under a CO_2 atmosphere. The bacteria were streaked onto petri dishes containing M8W medium until pure cultures were obtained. They were then transferred to individual Hungate tubes containing an agar slope of M8W medium, incubated for 24 to 48 h, and stored at −80°C until analysis. The bacteria were identified by standard microbiological methods, including Gram staining after growth in liquid M8G medium (M8 medium supplemented with 0.2% [wt/vol] glucose and 20% [vol/vol] rumen fluid) for 4 h at 35°C; determination of spore formation and motility in liquid M8G medium, substrate utilization patterns, and morphology; and identification of acidic fermentation products (14, 18, 27, 35). On the basis of the observed characteristics, genus or family names were assigned for most of the different isolates, placing them in what we consider an appropriate position in the existing classification. Substrate utilization patterns were determined by the techniques of Orpin et al. (29) with solidified M8 medium containing 10% (vol/vol) rumen fluid and solidified M8 medium supplemented with 0.2% (wt/vol) concentrations of the following carbohydrates (each in separate petri dishes): NAG, glycogen, maltose, starch, galactose, glucose, mannitol, arabinose, xylose, and sucrose. Bacterial growth relative to that in M8 medium lacking carbohydrates was determined after incubation for 24 to 48 h at 35°C. Aerobic growth was tested on Nutrient Agar (Difco Laboratories, Detroit, Mich.) at 35°C. The concentrations of VFAs, lactate, and succinate were determined by gas-liquid chromatography (36). Bacterial isolates were grown for 24 h in Hungate tubes containing 9 ml of liquid M8G medium. One milliliter of this culture was inoculated into another 9 ml of liquid M8G medium for an additional 24-h incubation period at 35°C. Fermentation products were determined after acidification of the liquid phase (36). Utilization of colloidal chitin was determined on solidified M8 medium containing 16% (vol/vol) colloidal chitin produced by a modification of the method of Lingappa and Lockwood (20). Colloidal chitin was prepared as follows. One gram of purified chitin from crab shells (catalog no. C 3641; Sigma) was dissolved in 30 ml of 50% H_2SO_4 at room temperature under constant stirring. The solution was filtered through a glass wool pad, and the chitin was precipitated in 0.5 liters of ice-cold distilled water. The pH was adjusted to 7.0 by addition of 10 M NaOH, and the solution was allowed to sediment for 24 h before the supernatant was removed. The chitin was then washed thoroughly and centrifuged for 10 min at 500 × *g* in a Sorvall GLC-2B centrifuge to form a colloidal solution, which was dissolved in distilled water to give a concentration of 10% and autoclaved at 115°C for 15 min. Chitobiase activity was tested as described by O'Brien and Colwell (26) by making replicas on Whatman no. 1 filter paper of colonies from cultures grown for 48 h in M8W medium.

SEM. Samples of food particles from the forestomach of herring-eating minke whales were fixed in 4% glutaraldehyde in 0.1 M Sørensen's phosphate buffer (pH 7.00). Preparation for SEM included postfixing in 1% OsO_4 for 1.5 h, dehydration in an ethanol series, and critical point drying in CO_2. The sample was glued to aluminum stubs with silver glue, sputter coated with gold, and examined in a JEOL JSM 840 scanning electron microscope.

RESULTS

Animals and sampling. The volumes of the in vivo forestomach contents ranged from 5 to 85 liters in the four minke whales examined, and all of the whales had fed on herring (Table 1). The pH of the forestomach contents ranged from 5.36 to 6.87 (Table 1). The DM in the forestomach contents ranged from 38.4 to 44.4% of the total contents in three of the minke whales (Table 1). Crude protein, lipids, and NH_4-N were found to constitute up to 39.7, 58.5, and 1.21% of the DM, respectively. Water-soluble carbohydrates were present in low concentrations, ranging from 1.74 to 2.46% of the DM. The sodium contents ranged from 1.58 to 2.34 g/kg of DM (Table 1) and were similar to that of the M8W medium.

Viable-cell counts. The total anaerobic bacterial population of the forestomach fluid from four minke whales, cultured in M8W medium, ranged from 73×10^7 to 145×10^8/ml of forestomach fluid (Table 1). Populations of lipolytic and NAG-using bacteria in one animal (whale 8-88) were enumerated by using a selective medium and constituted 89.7 and 95.2% of the viable population, representing $(13.0 \pm 1.4) \times 10^9$ and $(13.8 \pm 3.0) \times 10^9$ cells per ml of forestomach fluid, respectively. The total viable populations of anaerobic bacteria in three freshly caught and homogenized herring cultured in M8W medium were 67.5 ± 25.0, 56.7 ± 29.0, and 95.0 ± 39.0 cells per gram of homogenized herring.

Identification of bacteria. Strains of *Lactobacillus*, *Eubacterium*, *Fusobacterium*, *Sarcina*, *Streptococcus*, and *Ruminococcus* were isolated from the forestomach fluid of two of the minke whales but constituted different percentages of the viable bacterial population (Table 2). In addition, strains of *Bacteroides*, the family *Bacteroidaceae*, *Peptostreptococcus*, and *Coprococcus* were isolated from one of the whales (Table 2). Tables 3 and 4 give the results of the morphological and biochemical characterization of the bacterial strains isolated from the forestomach fluid. None of the bacterial strains utilized colloidal chitin or had chitobiase activity. Only 2.6% of 116 bacterial isolates could utilize NAG, 31.3% of 115 could utilize maltose, 82.0% of 116 could utilize starch, 12.8% of 117 could utilize galactose, 58.6% of 116 could utilize glucose, and 15.5% of 116 could utilize sucrose.

Bacteria isolated from fresh, homogenized herring were mainly of the genera *Pediococcus* and *Aerococcus* (Table 2). *Lactobacillus*, *Streptococcus*, and *Bacteroides* strains were found in the herring, but these strains were different from those isolated from the forestomachs of the minke whales (Tables 3 to 6). Strains of *Acetobacterium*, *Clostridium*, and the families *Enterobacteriaceae* and *Veillonellaceae* were also isolated. Morphological and biochemical characteristics of the bacterial strains isolated from freshly caught and homogenized herring are presented in Tables 5 and 6. None of the bacterial strains isolated from the herring were able to utilize colloidal chitin, but 17.2% of 64 strains had chitobiase activity. Seventy-one strains were tested for the ability to utilize various other substrates. NAG was utilized by 64.8% of the strains, maltose was utilized by 93.0%, starch was utilized by 78.9%, galactose

TABLE 2. Bacterial populations isolated from the forestomach fluid of herring-eating minke whales and from freshly caught and homogenized herring

Organism[a]	% of viable anaerobic bacterial population				
	Whale no.		Herring no.		
	2-90 (53)[b]	3-90 (64)	1 (26)	2 (20)	3 (26)
Lactobacillus spp.	37.7	4.7	7.7	—[c]	15.4
Acetobacterium spp.	—	—	—	—	3.8
Clostridium spp.	—	—	—	—	7.7
Enterobacteriaceae	—	—	3.8	5.0	—
Eubacterium spp.	17.0	1.6	—	—	—
Fusobacterium spp.	1.9	6.2	—	—	—
Bacteroides spp.	—	1.6	—	—	7.7
Bacteroidaceae	—	3.1	—	—	—
Sarcina spp.	3.8	14.1	—	—	—
Streptococcus spp.	28.3	42.2	19.2	—	11.5
Peptostreptococcus spp.	—	1.6	—	—	—
Ruminococcus spp.	7.5	17.2	—	—	—
Coprococcus spp.	—	6.2	—	—	—
Pediococcus spp.	—	—	23.1	35.0	34.6
Aerococcus spp.	—	—	26.9	45.0	7.7
Veillonellaceae	—	—	3.8	—	—
Others	3.8	1.6	15.4	15.0	11.5

[a] Characteristics of the bacterial strains are presented in Tables 3 to 6.
[b] Numbers in parentheses are numbers of strains isolated from each animal.
[c] —, not detected.

was utilized by 67.6%, glucose was utilized by 97.2%, and sucrose was utilized by 93.0%.

SEM. Food particles from the forestomachs of herring-eating minke whales were examined by SEM. Bacteria of different morphologies were found attached to food particles, e.g., on the collagen between the vertebrae (Fig. 1). Some of the bacteria were surrounded by a mass of tangled fibers of polysaccharides or branching sugar molecules extending from the bacterial surface and forming a glycocalyx which allowed them to stick to particles (Fig. 1).

DISCUSSION

Minke whales use baleen to filter food from seawater and do not masticate their food before swallowing it. The forestomach contents are, however, known to vary in composition from undigested fish to very liquid matter consisting of fish remnants, indicating that digestion is initiated in the forestomach. There are no glandular cells in the forestomach wall, which consists of a keratinized stratified squamous epithelium, and hence the decomposition of food which occurs in the forestomach cannot be due to the activity of secreted enzymes (28). A high concentration of anaerobic bacteria was observed in all animals examined (Table 1). Furthermore, the median concentration of major VFAs in minke whale forestomachs (n = 8; range in parentheses) was 94 (49 to 486) mM shortly after death; the VFAs consisted of acetate (63%), propionate (13%), and butyrate (24%) (22, 27a). In addition, isobutyric acid and isovaleric acid were present at median concentrations of 0.97 mM (range, 0.87 to 1.95 mM) and 2.07 mM (range, 1.68 to 3.19 mM), respectively, in minke whale forestomachs (n = 5) (27a). The contribution of VFAs to the daily energy requirement in minke whales is still unknown, but the high concentrations of anaerobic bacteria and VFAs and forestomach pHs between 5.4 and 6.9 (Table 1) are suggestive of microbial fermentation. This is also in accordance with observations from larger baleen whales such as grey whales (*Eschrichtius robustus*), bowhead whales (*Balaena mysticetus*), and fin whales (*Balaenoptera physalus*) (12, 13). The quality and the quantity of the substrate available for fermentation and the time elapsed between meals probably influence the number of bacteria, as is the case in ruminants (19). This may explain the variation in the total viable bacterial population between animals (Table 1). The forestomach bacterial counts were comparable to those of larger baleen whales (12, 13) and those of ruminants (17). The microbial population in the rumen of ruminants aids in utilization of dietary structural carbohydrates such as cellulose and hemicellulose, which constitute a significant proportion of the plants eaten, in such a way that the maximal amount of metabolizable energy can be obtained. Baleen whales, however, feed on fish and pelagic crustaceans (24), which contain mostly proteins and lipids but also polysaccharides (4, 11, 37) (Table 1). These are all substrates which may be degraded by fermentation. Thus, in comparisons of whales and ruminants, the substrate represents the most obvious difference in selecting for different bacterial strains in the minke whale forestomach and the bovine rumen. A rumen-type carbohydrate medium (M8W medium) was used for comparative purposes in this study. Using this medium, we found that the dominant bacterial strains isolated from the forestomach fluid of minke whales which had eaten a diet consisting solely of herring (Tables 2 to 4) belong to bacterial genera also isolated from the bovine rumen (17, 27, 35). This is probably due to similarities between the two habitats, including such features as temperature (35°C in the minke whale forestomach and 39°C in the bovine rumen), pH, and anaerobic conditions. Seawater seems to have little effect on the forestomach milieu. Even though it might be expected that seawater may enter the stomach together with the prey when swallowed, the concentrations of sodium and chloride in minke whale forestomachs (Table 1) were lower than those found in seawater (30) and even lower than those found in domestic ruminants (21).

Examinations of the bacterial population of one minke whale (no. 8-88) revealed large numbers of lipolytic bacteria in the forestomach when a selective lipolytic medium was used. This is consistent with the high lipid contents in the forestomach (Table 1). We did not enumerate the proteolytic bacteria in this initial work. The presence of branched-chain VFAs in the minke whale forestomach (27a) might, however, indicate the occurrence of protein fermentation (23). The low concentration of water-soluble carbohydrates in the minke whale forestomach (Table 1) is not surprising, because they are easily fermentable. Utilization of several different carbohydrates by different strains was determined (Tables 3 and 4). The numbers of NAG-using bacteria isolated from the forestomachs of whales 2-90 and 3-90 with M8W medium were found to be low (2.6% of the viable population). With a selective NAG medium containing 0.5% NAG as the sole carbohydrate and twice as much rumen fluid, the NAG-using bacteria constituted as much as 95.2% of the viable population in the forestomach of whale 8-88. This could be due to differences in carbohydrate concentrations in the different media. NAG is a component of chitin, which is found in the exoskeletons of crustaceans, which are also eaten by minke whales (24). Enzymatic hydrolysis of chitin to free NAG is performed by the chitinolytic system, which consists of two hydrolases which act consecutively. Chitinase hydrolyzes the polymers of NAG, while chitobiase hydrolyzes chitobiose and triose. We were not able to isolate any anaerobic chitinolytic bacteria from the forestomachs of herring-eating minke whales, nor did we find that any of the bacterial strains isolated from the dominant bacterial population had chitobiase activity. However, in krill-eating minke whales, anaerobic bacteria that

TABLE 3. Morphological and biochemical characteristics of strictly anaerobic bacteria isolated from the forestomach fluid of minke whale 2-90

Parameter	Result for indicated organism[a] (no. of isolates)									
	Lactobacillus strain L1 (20)	*Eubacterium* spp.		*Fusobacterium* strain F1 (1)	*Sarcina* strain S1 (2)	*Streptococcus* spp.		*Ruminococcus* strain R1 (4)	Unidentified strains	
		E1 (6)	E2 (3)			St1 (1)	St2 (14)		X1 (1)	X2 (1)
Characteristics										
Cell morphology	Coccoid rods in chains	Irregular rods, single or in pairs	Slim rods	Irregular rods	Large cocci in cuboidal packets	Irregular cocci in chains	Cocci in chains	Small cocci in chains	Coccoid regular rods in chains	Coccoid regular rods in chains
Gram stain	+	+	+	−	+	+	+	+	+	+
Spores	−	−	−	−	−	−	−	−	−	−
Motility	+	−	−	−	−	−	−	−	+	+
Utilization of:										
Glucose	−	d	−	−	d	+	d	d	+	+
NAG	−	−	−	+	−	−	−	−	−	+
Glycogen	−	d	−	−	−	−	−	−	−	+
Maltose	d	+	−	+	d	+	d	−	−	+
Starch	+	+	+	+	+	+	+	−	+	+
Galactose	−	d	−	−	+	−	−	−	−	+
Mannitol	−	−	−	−	−	−	d	−	−	−
Arabinose	−	d	−	−	−	−	−	−	−	−
Xylose	−	−	d	−	−	−	d	−	−	−
Sucrose	d	d	d	−	d	−	d	−	−	−
Fermentation products[b]	L, a, (s)	B/b, a, (l, s)	(a, s)	b, p	a, b, (s, p)	l, (a)	**L**/l, a, (s)	(a, s)	a, l, (p, b)	a, (l, s)

[a] +, ≥90% of strains positive; −, ≤10% of strains positive; d, 11 to 89% of strains positive.

[b] VFAs produced when bacteria were grown in liquid M8G medium for 24 h are listed in order of concentration from highest to lowest. Abbreviations: a, acetate; p, propionate; b, butyrate; l, lactate; s, succinate. Boldface capital letters represent concentrations of product greater than or equal to 1,500 μg/ml, lightface capital letters represent concentrations between 500 and 1,500 μg/ml, lowercase letters represent concentrations between 100 and 500 μg/ml, and lowercase letters in parentheses represent concentrations of less than 100 μg/ml. Slashes indicate differences in production of individual VFAs between isolates of the same strain.

TABLE 4. Morphological and biochemical characteristics of strictly anaerobic bacteria isolated from the forestomach fluid of minke whale 3-90

Parameter	Result for indicated organism[a] (no. of isolates)																
	Lactobacillus spp.			*Eubacterium* strain E3 (1)	*Fusobacterium* strain F2 (4)	*Bacteroides* strain B1 (1)	*Bacteroidaceae* strain B2 (2)	*Sarcina* strain S2 (9)	*Streptococcus* spp.			*Peptostreptococcus* strain P1 (1)	*Ruminococcus* spp.		*Coprococcus* spp.		Unidentified strain X3 (1)
	L2 (1)	L3 (1)	L4 (1)						St3 (1)	St4 (14)	St5 (12)		R2 (6)	R3 (5)	C1 (1)	C2 (3)	
Characteristics																	
Cell morphology	Slim rods	Irregular rods, single or in pairs	Irregular rods, single or in pairs	Irregular rods	Irregular rods	Irregular rods	Slim rods	Large cocci in cuboidal packets	Irregular cocci in chains	Cocci in chains	Cocci in chains	Cocci in chains	Small cocci in chains	Small cocci in chains	Cocci in chains	Small cocci in chains	Slim, regular rods in pairs
Gram stain	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	+
Spores	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Motility	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−
Utilization of:																	
Glucose	+	+	+	+	+	+	+	d	+	+	d	+	+	d	+	+	+
NAG	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Glycogen	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	+
Maltose	−	−	−	−	d	−	−	−	−	+	−	−	−	−	−	−	+
Starch	−	+	+	−	d	−	d	d	−	+	+	+	+	d	+	+	+
Galactose	+	+	+	−	−	−	d	−	−	−	d	−	−	−	−	−	−
Mannitol	−	+	+	+	d	−	d	d	+	d	d	+	−	d	−	+	+
Arabinose	+	−	+	−	−	−	−	d	−	−	−	−	−	d	+	+	+
Xylose	+	−	−	−	−	−	−	d	−	d	−	−	−	d	+	+	−
Sucrose	−	−	−	−	d	−	d	−	−	d	−	−	−	−	−	−	−
Fermentation product(s)[b]	**L**, a, (s)	l, a, (s)	L, a, p, (b)	(a, s)	B, p/(p), (s)	a, l, (s)	(s)	a, b/(b), (s)	l, (a)	**L**/L, a, (s)	**L**/l, a, (s)	A, ib, iv, (l, v, ic, s)	A/(a), l/(l), (s)	(a, s)	b, l, (a, s)	b, (a)	A, ib, (v, l, iv, b, s)

[a] +, ≥90% of strains positive; −, ≤10% of strains positive; d, 11 to 89% of strains positive.

[b] VFAs produced when bacteria were grown in liquid M8G medium for 24 h are listed in order of concentration from highest to lowest. Abbreviations: a, acetate; p, propionate; b, butyrate; l, lactate; s, succinate; iv, isovalerate; ib, isobutyrate; ic, isocaproate. Boldface capital letters represent concentrations of product greater than or equal to 1,500 μg/ml, lightface capital letters represent concentrations between 500 and 1,500 μg/ml, lowercase letters represent concentrations between 100 and 500 μg/ml, and lowercase letters in parentheses represent concentrations of less than 100 μg/ml. Slashes indicate differences in production of individual VFAs between isolates of the same strain.

TABLE 5. Morphological and biochemical characteristics of bacteria isolated from freshly caught and homogenized herring (n = 3)

Parameter	Result for indicated organism (no. of isolates)															
	Lactobacillus spp.				*Aceto-bacterium* strain H-A (1)	*Clostridium* strain H-C (2)	*Enterobacteriaceae*		*Bacteroides* spp.		*Streptococcus* spp.					
	H-L1 (2)	H-L2 (1)	H-L3 (2)	H-L4 (1)			H-E1 (1)	H-E2 (1)	H-B1 (1)	H-B2 (1)	H-St1 (2)	H-St2 (1)	H-St3 (1)	H-St4 (1)	H-St5 (1)	H-St6 (2)
Characteristics[a]																
Cell morphology	Irregular rods	Short rods	Coccoid rods	Coccoid rods	Rods	Rods	Rods	Rods	Irregular rods	Irregular rods	Cocci in chains	Small cocci in chains	Cocci in long chains	Cocci in long chains	Cocci in chains	Cocci, single or in pairs
Gram stain	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+
Spores	−	−	−	−	−	+ (es)	−	−	−	−	−	−	−	−	−	−
Motility	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−
Facultatively anaerobic	−	−	−	−	−	−	+	+	−	−	−	−	+	+	+	+
Strictly anaerobic	+	+	+	+	+	+	−	−	+	+	+	+	−	−	−	−
Utilization of[a]:																
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NAG	+	+	+	+	−	d	+	+	−	−	+	−	+	+	+	−
Glycogen	−	−	−	−	+	d	−	−	−	−	−	+	−	−	−	−
Maltose	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Starch	+	+	+	+	−	−	+	+	+	+	+	−	+	+	+	+
Galactose	+	+	+	+	−	−	−	−	−	−	d	−	−	+	+	+
Mannitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Arabinose	−	−	−	−	−	d	−	−	−	−	−	−	−	−	−	−
Xylose	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Sucrose	+	+	+	+ (w)	+	d	+	+	+	+	+	−	+ (p)	+	+	+
Production of chitobiase[b]	+	−	+	−	ND	d	+	−	−	−	−	−	−	ND	−	−
Fermentation product(s)[c]	L, a	l, a	L	L, a	a, (iv)	a, iv, (ib)	l, a	a, (iv, ib)	A, ic, ib, (iv, v, l)	A, (iv, ib)	L/l, a	(l, a)	l, (a)	L	L, a, (b)	l, (a)

[a] +, ≥90% of strains positive; −, ≤10% of strains positive; d, 50% of strains positive; p, pigmented (yellow); w, white; es, endospores.

[b] Reactions were graded as weakly positive (+), or negative (−), depending on fluorescence intensity when the bacterial culture was exposed to UV light at 302 nm. d, 50% of strains positive; ND, not determined.

[c] VFAs produced when bacteria were grown in liquid M8G medium for 24 h are listed in order of concentration from highest to lowest. Abbreviations: a, acetate; ib, isobutyrate; b, butyrate; iv, isovalerate; v, valerate; ic, isocaproate; l, lactate. Boldface capital letters represent concentrations of product greater than or equal to 1,500 μg/ml, lightface capital letters represent concentrations between 500 and 1,500 μg/ml, lowercase letters represent concentrations between 100 and 500 μg/ml, and lowercase letters in parentheses represent concentrations of less than 100 μg/ml. Slashes indicate differences in production of individual VFAs between isolates of the same strain.

TABLE 6. Morphological and biochemical characteristics of unidentified bacteria isolated from freshly caught and homogenized herring (n = 3)

Parameter	Result for indicated organism (no. of isolates)																		
	Pediococcus spp.						*Aerococcus* spp.			*Veillonellaceae*, strain H-V (1)	Unidentified strains								
	H-P1 (5)	H-P2 (2)	H-P3 (2)	H-P4 (3)	H-P5 (1)	H-P6 (9)	H-A1 (1)	H-A2 (1)	H-A3 (16)		H1 (1)	H2 (1)	H3 (1)	H4 (1)	H5 (2)	H6 (1)	H7 (1)	H8 (1)	H9 (1)
Characteristics[a]																			
Cell morphology	Cocci in pairs or tetrads	Cocci in pairs or tetrads	Cocci in pairs or tetrads	Cocci in pairs or tetrads	Cocci in pairs or tetrads	Cocci in pairs or tetrads	Cocci in pairs or tetrads	Cocci in pairs or tetrads	Cocci in pairs or tetrads	Cocci in chains	Cocci in chains	Cocci in pairs, tetrads, or clusters	Rods, single or in chains	Irregular rods	Irregular rods	Irregular, slim rods	Small rods in chains	Small rods in chains	Small rods
Gram stain	+	+	+	+	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−
Spores	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Motility	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−
Facultatively anaerobic	−	+ (p)	+ (w)	+	+ (w)	+	+	+	+	−	+	+	−	−	−	−	+	+	+
Strictly anaerobic	+	−	−	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	−
Utilization of[a]:																			
Glucose	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NAG	d	+	−	+	ND	+	−	+	+	+	+	−	+	−	+	−	+	+	−
Glycogen	−	−	−	−	−	d	+	−	−	−	−	+	−	−	−	−	−	−	−
Maltose	+	d	d	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+
Starch	+	−	−	d	−	+	−	+	+	−	+	−	−	+	+	+	+	−	−
Galactose	+	−	−	d	−	+	+	+	+	−	+	−	−	+	d	+	+	−	−
Mannitol	−	−	−	−	−	d	−	−	−	−	−	−	+	−	+	+	−	−	−
Arabinose	−	−	−	−	−	d	−	−	−	−	−	−	−	−	d	+	−	−	−
Xylose	−	−	−	d	−	d	−	−	d	−	−	−	−	−	−	−	−	−	+
Sucrose	+	+	+	+	−	+	+	+	+	+	+	−	−	+	+	+	+	+	−
Production of chitobiase[b]	−	−	−	−	−	d	−	−	−	−	−	−	−	−	d	−	++	+	−
Fermentation product(s)[c]	**L/l**	**L**	**L**	**L**	l, (a, b)	l	a, (iv)	a, iv, (ib, l)	(a)	l	a, l	l, (a)	s	NP	NP	a	(l)	l, (a)	l, (s)

[a] +, ≥90% of strains positive; −, ≤10% of strains positive; d, 11 to 89% of strains positive; p, pigmented (yellow); w, white; ND, not determined.

[b] Reactions were graded as positive (++), weakly positive (+), or negative (−), depending on fluorescence intensity when the bacterial culture was exposed to UV light at 302 nm. d, 11 to 89% of the strains were positive.

[c] VFAs produced when bacteria were grown in liquid M8G medium for 24 h are listed in order of concentration from highest to lowest. Abbreviations: a, acetate; b, butyrate; l, lactate; iv, isovalerate; ib, isobutyrate; NP, none produced. Boldface capital letters represent concentrations of product greater than or equal to 1,500 μg/ml, lightface capital letters represent concentrations between 500 and 1,500 μg/ml, lowercase letters represent concentrations between 100 and 500 μg/ml, and lowercase letters in parentheses represent concentrations of less than 100 μg/ml. Slashes indicate differences in production of individual VFAs between isolates of the same strain.

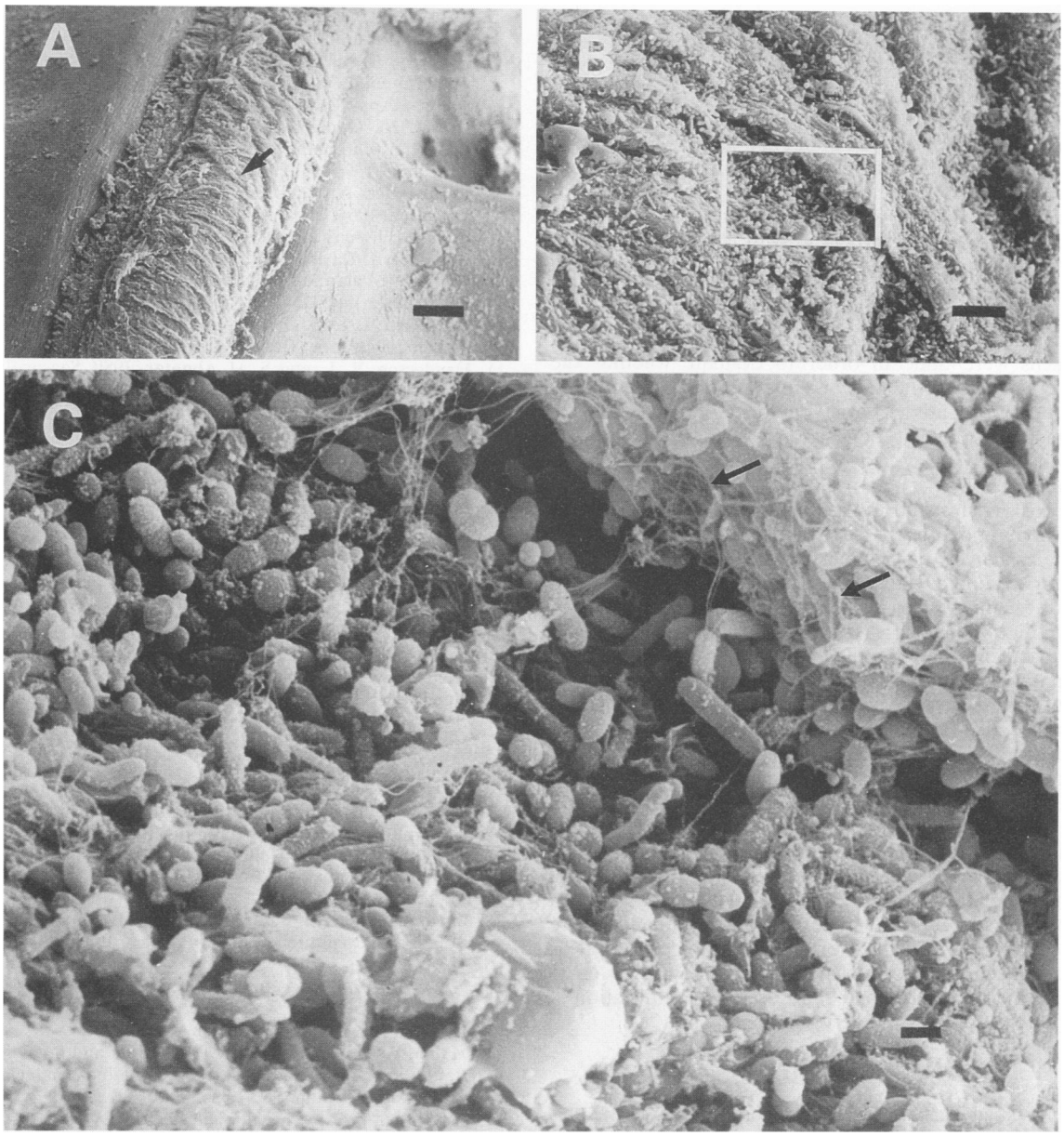

FIG. 1. Scanning electron micrograph of the cartilage between two herring vertebrae from the forestomach of a minke whale. (A) Close-up of the herring cartilage. Bar = 100 μm. (B) Magnified area of the cartilage, identified with an arrow in panel A, showing large numbers of bacteria attached to the herring particle. Bar = 10 μm. (C) The area outlined by the rectangle in panel B was examined at a higher magnification, showing bacteria with different morphologies, some surrounded by a glycocalyx (arrows). Bar = 1 μm.

hydrolyze chitin have been isolated from the forestomach fluid (21a). Even though we did not succeed in isolating chitinolytic and chitobiase-producing bacteria from herring-eating minke whales, such bacteria might be present at lower concentrations, making utilization of chitin possible if the whales switch prey and start feeding on krill.

The concentration of bacteria in fresh homogenized herring was much lower than that found in the forestomach fluid of herring-eating minke whales (Table 1). Furthermore, bacterial strains isolated from freshly homogenized herring were all found to be different from those isolated from the dominant bacterial population in the forestomach fluid of the minke whales (Tables 3 to 6). Strains from some of these genera, such as strains of *Lactobacillus*, which grow even at temperatures close to freezing, have been isolated from herring (32), while a marine, psychrophilic bacterium similar to members of the *Bacteroidaceae* has been isolated from capelin (7), and clostridia have been found in the intestines of haddock (*Gadus aeglefinus*) (33). These strains described earlier (7, 32, 33) are also different from those isolated from minke whale forestomachs (Tables 3 and 4). The strains which were isolated from the herring in this study were facultatively (61.1%) or obligately (38.9%) anaerobic and able to grow at 35°C. In the marine fish *Enophrys bison*, chitinolytic activity in the stomach was reported to be produced by bacteria (9). We did not isolate any chitinolytic bacteria from herring, but chitobiase activity

was observed in some strains (Tables 5 and 6). Alone, chitobiase could be considered a functionless enzymatic remnant of the chitinolytic system, or it may function by hydrolyzing other molecules that contain NAG dimers, e.g., glycoproteins and mucopolysaccharides.

This study has shown that the dominant bacterial population in the forestomachs of herring-eating minke whales is different from that found in their prey, and we believe that this dominant population is an indigenous population. This is not surprising given the difference in growth conditions, such as temperature, pH, and salinity. The temperature of seawater in the northern Atlantic Ocean is below 10°C, and the pH of seawater usually ranges between 7.5 and 8.5 (30).

Electron microscopic examinations of food particles from forestomachs were done in order to evaluate whether the bacteria actually attack the prey and not only live in the fluid as documented by counting. Large numbers of bacteria were found attached to herring bones; some of the bacteria were surrounded by a glycocalyx (Fig. 1) which enabled them to stick to food particles (5).

On the basis of this study, it is concluded that an indigenous bacterial population in the forestomachs of herring-eating minke whales actively participates in digestion of prey. Digestion of prey in minke whales has been studied by an in vitro digestibility technique, and it was found that the disappearance of DM and digestible energy (the amount of energy in the prey assumed to be absorbed by the whale) of herring were 80.4% (n = 18 parallels) and 92.1% (n = 16 parallels), respectively, after a 36 h incubation period (25). The highest rate of DM disappearance occurred when simulating the forestomach microbial digestion (25). Bacterial digestion in ruminants is accomplished by loss of energy as ammonia and methane, the latter of which is eructed. This study indicates that in minke whales, a large forestomach population of bacteria contributes to digestion. Even though the relative size of the minke whale forestomach is small in comparison with the rumen (28), compartmentalization of the minke whale stomach system increases retention of food in the forestomach and hence increases the time available for microbial digestion. It is evident that bacterial breakdown of food followed by enzymatic action in the fundic chamber ensures better utilization of the food before it enters the very short intestine (28).

ACKNOWLEDGMENTS

We extend special thanks to Wenche Sørmo, Arnoldus Schytte Blix, and Erling S. Nordøy for assistance in the field and the whaling crews aboard the M/V *Ann Brita* and M/V *Isqueen* for their cooperation. Colin G. Orpin is acknowledged for constructive discussions during the planning of this project.

This study was supported by the Norwegian Fisheries Research Council (grant 4001-408.007).

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