FULL PAPER *Anatomy*

Ultrastructural study on the morphological changes in indigenous bacteria of mucous layer and chyme throughout the rat intestine

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ABSTRACT. Indigenous bacteria in the alimentary tract are exposed to various bactericidal peptides and digestive enzymes, but the viability status and morphological changes of indigenous bacteria are unclear. Therefore, the present study aimed to ultrastructurally clarify the degeneration and viability status of indigenous bacteria in the rat intestine. The majority of indigenous bacteria in the ileal mucous layer possessed intact cytoplasm, but the cytoplasm of a few bacteria contained vacuoles. The vacuoles were more frequently found in bacteria of ileal chyme than in those of ileal mucous layer and were found in a large majority of bacteria in both the mucous layer and chyme throughout the large intestine. In the dividing bacteria of the mucous layer and chyme throughout the intestine, the ratio of area occupied by vacuoles was almost always less than 10%. Lysis or detachment of the cell wall in the indigenous bacteria was more frequently found in the large intestine than in the ileum, whereas bacterial remnants, such as cell walls, were distributed almost evenly throughout the intestine. In an experimental control of long-time-cultured *Staphylococcus epidermidis* on agar, similar vacuoles were also found, but cell-wall degeneration was never observed. From these findings, indigenous bacteria in the mucous layer were ultrastructurally confirmed to be the source of indigenous bacteria in the chyme. Furthermore, the results suggested that indigenous bacteria were more severely degenerated toward the large intestine and were probably degraded in the intestine.

KEY WORDS: indigenous bacteria, intestine, rat, transmission electron microscopy

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Numerous indigenous bacteria settle in the animal alimentary tract. The bacterial settlement initiates immediately after birth by bacteria derived from mother, and the number and composition of indigenous bacteria are stable in the adult alimentary tract [3]. The settlement of indigenous bacteria is regulated by various factors from their hosts: the rapid migration of epithelial cells, acceleration of epithelial cell proliferation, physical elimination by epithelial cells, digestive enzymes, bactericidal peptides and so on [14, 15, 18, 21, 22, 35, 36]. In the animal alimentary tract, lysozyme, soluble phospholipase A2 (sPLA2), α-defensin and β-defensin-1 and -2 might regulate the settlement of indigenous bacteria [27, 35, 36]. The lysozyme hydrolyzes the β-(1,4)-glycosidic bond between the alternating N-acetylmuramic acid and N-acetylglucosamine residues of peptidoglycan [6, 9], sPLA2 hydrolyzes the phospholipid component of the bacterial cell membrane [31], and defensin forms voltage-dependent channels in the planar lipid bilayer membrane [16] and increases the permeability of the bacterial membrane [17]. However, how these bactericidal peptides cooperatively exert their bactericidal effects against the indigenous bacteria has been never clarified *in vivo*.

The population of bacteria is regulated not only by their hosts, but also by the bacteria themselves. Bacteria can produce diffusible signal molecules, known as "autoinducers" (AI), so that the AI concentration in the milieu increases in association with the growth of bacterial colonies. When the AI concentration reaches a threshold, AI can alter the pattern of gene expression in bacteria. AI-mediated regulation of gene expression is called "quorum sensing" [1, 2]. The gene expression regulated by quorum sensing includes the production of bacteriocins, which are toxic to bacteria, closely related to the producing bacteria and thought to be widespread among eubacteria [7, 20, 24]. Therefore, indigenous bacteria in the animal alimentary tract might receive a bactericidal effect by antibiotics or bacteriocins that the bacteria themselves produce, in addition to host-derived bactericidal peptides. However, the viability status

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and morphological changes of indigenous bacteria in the intestine are unclear, so the fate of indigenous bacteria in the chyme is still unknown. Therefore, the present study aims to ultrastructurally clarify the degeneration and viability status of indigenous bacteria in the mucous layer and the chyme in the rat intestine. In the present study, rat intestinal segments, except for the duodenum and jejunum, were used as observation subjects; consistent with a previous study [33], sufficient bacteria were not observed in the duodenum and jejunum in our preliminary observation.

MATERIALS AND METHODS

Animals: Five male Wistar rats aged 7 weeks (Japan SLC Inc., Hamamatsu, Japan) were maintained under conventional laboratory housing conditions. They were permitted free access to water and food (Lab MR Stock, Nosan Corp., Yokohama, Japan). The animal facility was maintained under conditions of a 12 hr light/dark cycle at 23 ± 1°C and 50–60% humidity. Clinical and pathological examinations in all animals confirmed no signs of disorder. This experiment was approved by the Institutional Animal Care and Use Committee (Permission number: 19-05-07 and 22-05-01) and carried out according to the Kobe University Animal Experimentation Regulations.

Tissue preparation: After euthanasia with an overdose peritoneal injection of pentobarbital sodium (Kyoritsu Seiyaku Corp., Tokyo, Japan), small tissue blocks and chyme were removed from the ileum, the apex of cecum, the ascending colon and the rectum of 5 rats. All tissue blocks were immersion-fixed in 2.5% glutaraldehyde-2.0% paraformaldehyde fixative in 0.1 M phosphate buffer (PB, pH 7.4) (GAPA) for 24 hr at 4°C. After postfixation with 1.0% OsO_4 in PB for 90 min at room temperature (r.t.), the small specimens were dehydrated and embedded in a Quetol 812-mixture.

Bacteria preparation: In order to compare the ultrastructural characteristics of the degeneration process of bacteria in chyme with those of bacteria that have proliferated without bactericidal substances, the cultured colonies of *Staphylococcus epidermidis* were also ultrastructurally investigated. Briefly, after cultivation of *S. epidermidis* on 7% NaCl heart infusion agar for 1 or 45 days at 37°C, bacterial colonies with agar were fixed with GAPA. After fixation, bacterial colonies were stripped from the agar surface and postfixed with 1.0% OsO_4 in PB for 2 hr at r.t. The specimens were dehydrated and embedded in a Quetol 812-mixture.

Transmission electron microscopy: Semithin sections with 1 μm in thickness were cut using an ultramicrotome (Sorvall MT-1, Dupont, Newton, CT, U.S.A.) and stained with 0.01 M phosphate-buffered 0.05% toluidine blue solution (pH 7.4). Intestinal samples with the settlement of indigenous bacteria were then chosen for transmission electron microscopy. Ultrathin sections with 70 nm in thickness were made by the same ultramicrotome and stained with both 4% uranyl acetate solution and lead citrate solutions by Sato's method modified by Hanaichi [13]. The ultrathin sections were observed under a transmission electron microscope (H-7100, Hitachi, Tokyo, Japan; and JEM-1400, JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 75 or 80 kV.

Quantitative ultrastructural observation: Thirty or more indigenous bacteria were chosen from 5 portions in the mucous layer or chyme of each intestinal segment, respectively. Then, indigenous bacteria with vacuoles in their cytoplasm were counted in each portion, and the mean relative frequency of bacteria with vacuoles was calculated in the mucous layer or chyme of each intestinal segment. The data were presented as means ± standard deviations (SDs).

Five-hundred indigenous bacteria were chosen from the mucous layer or chyme of each intestinal segment, respectively. Then, the number of indigenous bacteria with the following characteristics was counted: 1) cell division, 2) lysis of the cell wall, 3) detachment of the cell wall from cell membrane, 4) formation of endospore and 5) death (remnants of the cell wall or cytoplasm). Furthermore, the ratio of area occupied by vacuoles in each bacterial protoplasm was estimated in all 500 bacteria, and the number of bacteria with each 10% vacuole occupancy was counted in the mucous layer or chyme of each intestinal segment, respectively.

Statistical analysis: Statistical analysis against the relative frequency of indigenous bacteria with vacuoles was performed with ANOVA with Scheffe's multiple comparison test for comparison in the chyme between the intestinal segments, and with the Mann-Whitney *U* test for comparison between the mucous layer and chyme in the ileum. *P* values less than 0.05 were considered statistically significant.

RESULTS

Indigenous bacteria in the mucous layer of rat intestine: Cocci and bacilli ordinarily existed in the mucous layer throughout the rat intestine. Additionally, segmented filamentous bacteria (SFB) were occasionally contained in the mucous layer of the ileum, but not in those of the cecum and ascending colon. Spiral-shaped bacteria were found in the mucous layer of the cecum and ascending colon, but not in that of the ileum. Indigenous bacteria in the mucous layer of the ascending colon contained numerous long and thick bacilli. These long and thick bacilli were also rarely found in the mucous layer of the cecum, but not in that of the ileum. The mucous layers of intestinal crypts of the ileum never contained bacteria, whereas those of the cecum were often packed with numerous spiral-shaped bacteria and a few bacilli, while those of the ascending colon rarely contained some kinds of bacteria, such as short bacilli and long and thick bacilli (Fig. 1a–1f).

In the ileal mucous layer, the majority of indigenous bacteria possessed intact cytoplasm, which was divided into two parts: cytoplasmic matrices with homogenous high electron density and nucleoid bodies with low electron density. On the other hand, in the mucous layers of the cecum and ascending colon, indigenous bacteria with intact cytoplasm were few, but vacuolated indigenous bacteria were numerously contained. These low-electron-density vacuoles

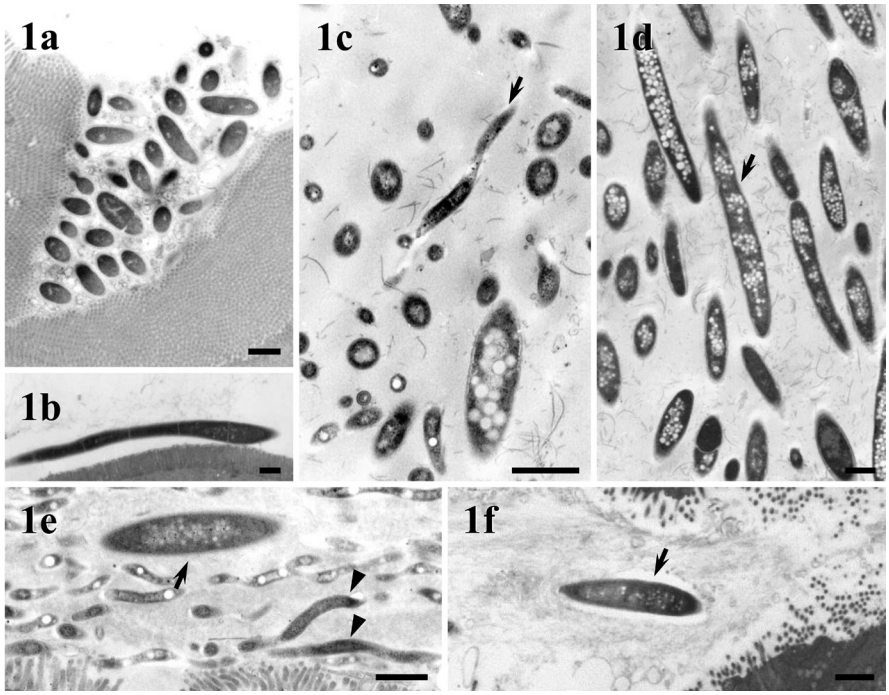

Fig. 1. a–d) Ultrastructure of indigenous bacteria in the mucous layer of the ileum (a, b), cecum (c) and ascending colon (d). a) Rod-shaped indigenous bacteria have intact cytoplasm with homogeneous electron density and nucleoid bodies in their cytoplasm in the ileal mucous layer on the epithelial surface. b) Segmented filamentous bacteria are visible in the ileal mucous layer. c, d) Coccal or rod-shaped indigenous bacteria have vacuoles in their cytoplasm in the mucous layer of the cecum (c) and ascending colon (d). Extremely slim spiral-shaped bacteria in the cecum (c, arrow) and long and thick bacilli in the ascending colon (d, arrow) are visible in each intestinal segment. e, f) In the intestinal crypts, spiral-shaped bacteria are numerously visible in the cecum (e, arrowheads), while a sole indigenous bacterium is visible in the ascending colon (f). The vacuolation of indigenous bacteria is lower degree in the intestinal crypt of the cecum (e, arrow) and ascending colon (f, arrow) than in the mucous layers of the cecum (c) and ascending colon (d). Bar=1 μm.

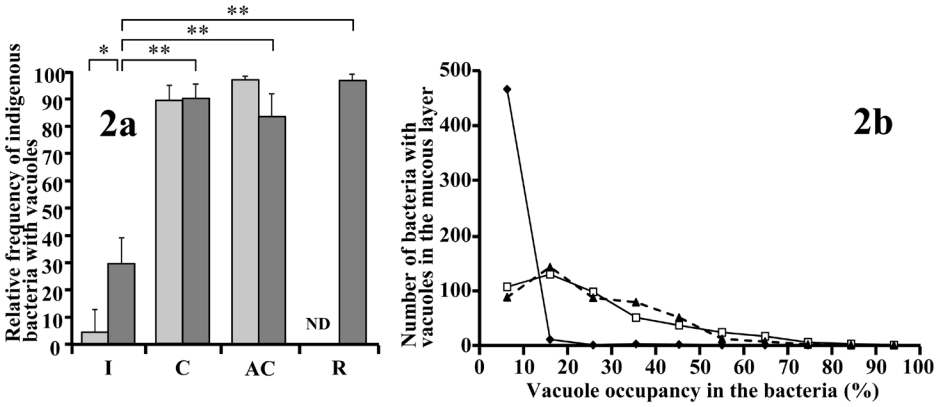

Fig. 2. a) The relative frequencies of indigenous bacteria with vacuoles in the mucous layer (light gray column) or chyme (dark gray column) in each intestinal segment. Asterisk, $P<0.05$. Double asterisks, $P<0.01$. I, ileum. C, cecum. AC, ascending colon. R, rectum. ND, not determined. b) The number of indigenous bacteria having each 10% vacuole occupancy in their cytoplasm in the mucous layer of the ileum (black diamond), cecum (white square) and ascending colon (black triangle).

were variously sized, were not surrounded by cell membrane and were found in almost all bacteria with different morphologies throughout the intestine. The ratio of area occupied by vacuoles in the bacterial cytoplasm was less than 10% in the ileal mucous layer, but more than 10% in those of the cecum and ascending colon (Figs. 1a–1d, 2a, 2b). In the

Table 1. Number of indigenous bacteria with various structural features in the rat intestine

Intestinal segment	Portion	Number of bacteria with each structural feature*				
		Cell division	Lysis of cell wall	Detachment of cell wall	Formation of endospore	Death
Ileum	Mucous layer	28	42	6	10	11
	Chyme	61	11	14	2	35
Cecum	Mucous layer	3	41	42	7	29
	Chyme	13	83	36	3	60
Ascending colon	Mucous layer	1	40	122	16	23
	Chyme	10	55	50	4	28
Rectum	Mucous layer	-	-	-	-	-
	Chyme	10	56	59	9	35

*; A total of 500 bacteria were measured in each sample.

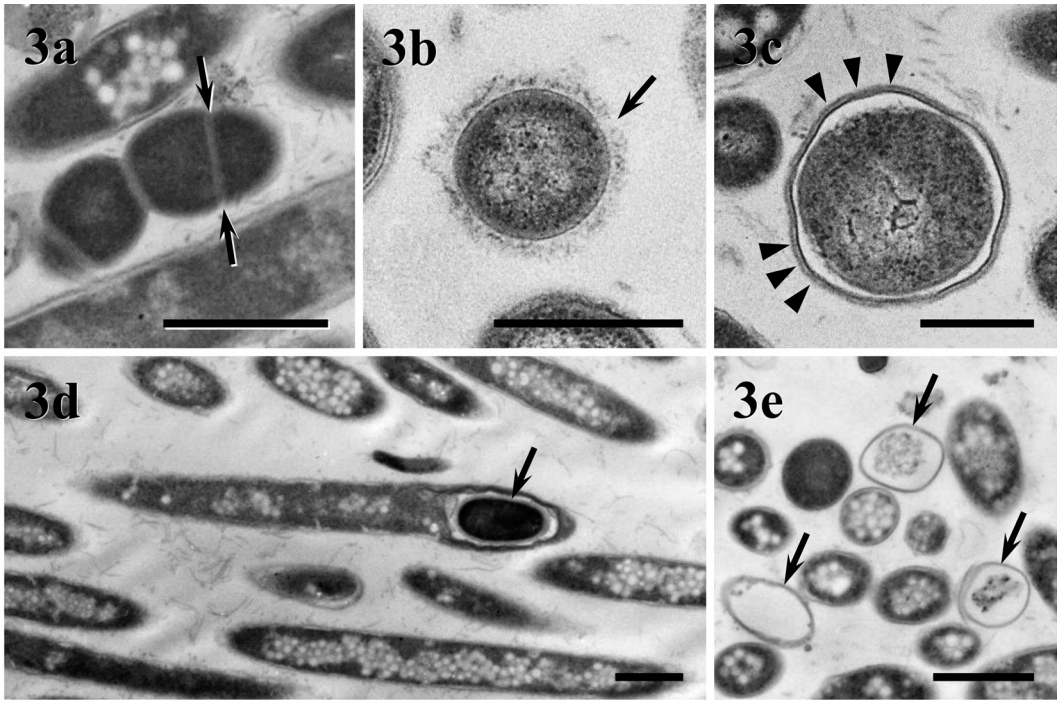

Fig. 3. Various morphological characteristics of indigenous bacteria: a) cell-dividing bacterium with septum (inter-arrows) in the cecal chyme, b) indigenous bacterium with lysis of the cell wall (arrow) in the cecal chyme, c) indigenous bacterium with detachment of the cell wall from the cell membrane (arrowheads) in the chyme of the ascending colon, d) indigenous bacterium with the endospore in its cytoplasm (arrow) in the mucous layer of the ascending colon, e) dead bacteria (arrows) in the cecal chyme. a, d, e) Bar=1 μm. b, c) Bar=500 nm.

cecum and ascending colon, highly vacuolated bacteria were frequently found in the mucous layer, but few were found in the intestinal crypts (Fig. 1c–1f).

The indigenous bacteria in the mucous layer throughout the intestine occasionally showed cell divisions (Fig. 3a). Cell-dividing bacteria possessed incomplete or complete septal walls between paired bacteria and were abundant in the ileum, but very rare throughout the large intestine (Table 1). The ratio of area occupied by vacuoles was almost always less than 10% in the dividing bacteria throughout the intestine.

Indigenous bacteria rarely possessed degenerated cell walls, such as those with lysis or detachment. The lysis of cell wall was observed as the cell wall with irregular thickness (Fig. 3b). The number of bacteria showing lysis of the cell wall in the mucous layer was approximately similar throughout the intestine. The number of bacteria with detached cell walls from the cell membrane was higher in the large intestine than in the ileum (Fig. 3c). Bacteria laden with endospore were rarely found in the mucous layer throughout the intestine (Fig. 3d, Table 1).

Dead bacteria existed as cell wall remnants, remnants

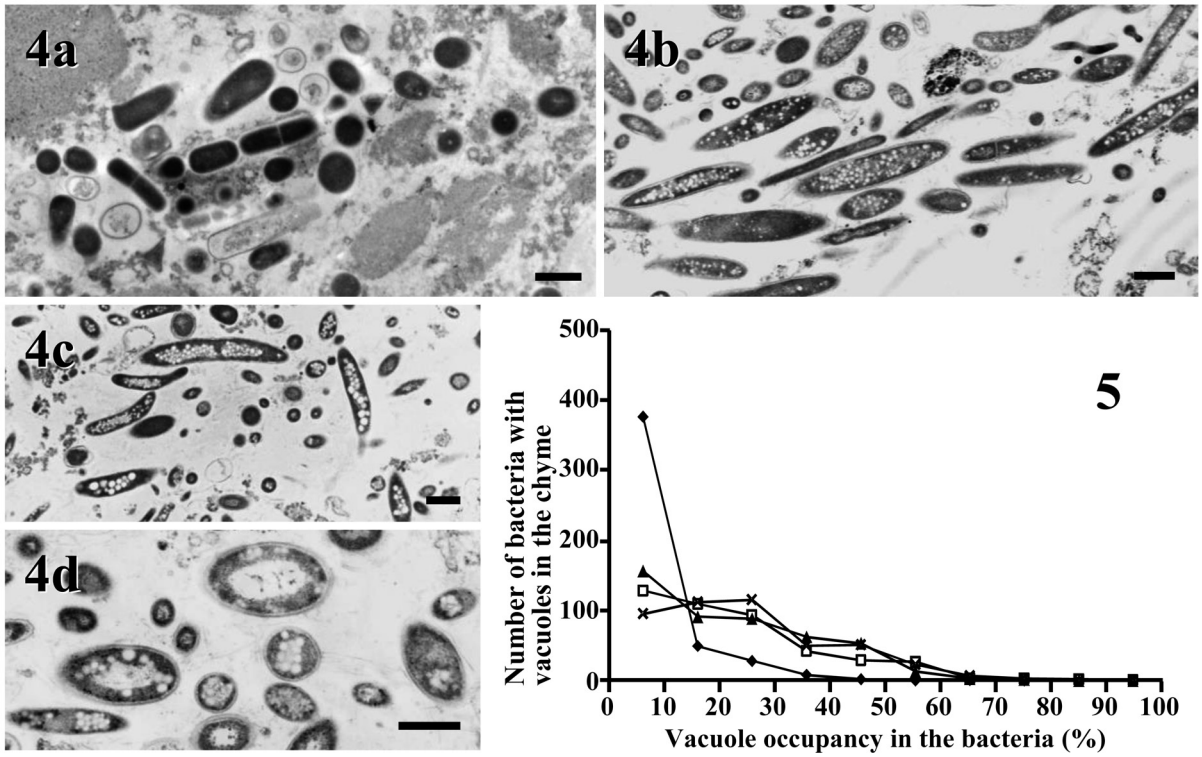

Fig. 4. Ultrastructure of indigenous bacteria in the chyme throughout the rat intestine. Various types of bacteria are visible, in the chyme of the ileum (a), cecum (b), ascending colon (c) and rectum (d). Many vacuolated bacteria are visible, especially in the chyme of the large intestine. Bar=1 μm.

Fig. 5. The numbers of indigenous bacteria having each 10% vacuole occupancy in their cytoplasm in the chyme of the ileum (black diamond), cecum (white square), ascending colon (black triangle) and rectum (black cross).

of both cell walls and partial protoplasms or cytoplasm remnants. Dead bacteria were slightly more abundant in the mucous layer of the cecum and ascending colon than in that of the ileum (Fig. 3e, Table 1). Most of the dead bacteria in the ileal mucous layer were found as cell wall remnants, whereas most of the dead bacteria in the cecal mucous layer were either remnants of cytoplasm or remnants of both cell walls and cytoplasm. In the mucous layer of the ascending colon, most of the dead bacteria were remnants of both cell walls and cytoplasm. In the mucous layer of the rectum, the ultrastructural characteristics of indigenous bacteria could not be determined, because indigenous bacteria were scarce there.

Indigenous bacteria in the chyme of rat intestine: In the chyme throughout the rat intestine, cocci and bacilli were constantly existed, and SFB were not found in the chyme throughout the intestine. Spiral-shaped bacteria and long and thick bacilli, which were found in the mucous layer of the cecum and ascending colon, were also found in the chyme of the cecum, ascending colon and rectum, but not in the ileal chyme.

Indigenous bacteria in the chyme throughout the intestine possessed vacuoles in their cytoplasm without relation to bacterial morphologies, although the frequency of vacuolated bacteria in the chyme differed among the intestinal segments (Fig. 4a–4d). The relative frequency of vacuolated bacteria in the chyme of the ileum was significantly lower than in that of the large intestine and larger than that in the mucous layer of ileum (Fig. 2a). The ratio of area occupied by vacuoles in the bacterial cytoplasm was low in the ileal chyme (mainly less than 30%), whereas indigenous bacteria with high ratio of vacuole area (more than 40%) were found in the chyme of the large intestine (Fig. 5).

Cell-dividing bacteria were more abundant in the ileal chyme than in that of the large intestine, and more abundant in the chyme than in the mucous layer of all intestinal segments. In the dividing bacteria in the chyme throughout the intestine, the ratio of area occupied by vacuoles was almost always less than 10%. Lysis or detachment of the cell wall in the chyme was more abundantly found in indigenous bacteria of the large intestine than in those of the ileum. There were very few bacteria laden with endospore in the chyme of all intestinal segments. Dead bacteria were more abundantly found in the chyme of the cecum than in that of the other intestinal segments (Fig. 3a–3e, Table 1). The dead bacteria were observed as cell wall remnants and remnants of both cell walls and cytoplasm throughout the intestine, whereas cytoplasm remnants without cell walls were rarely found in the chyme of the cecum, ascending colon and rectum, but were not found at all in that of the ileum. Dead

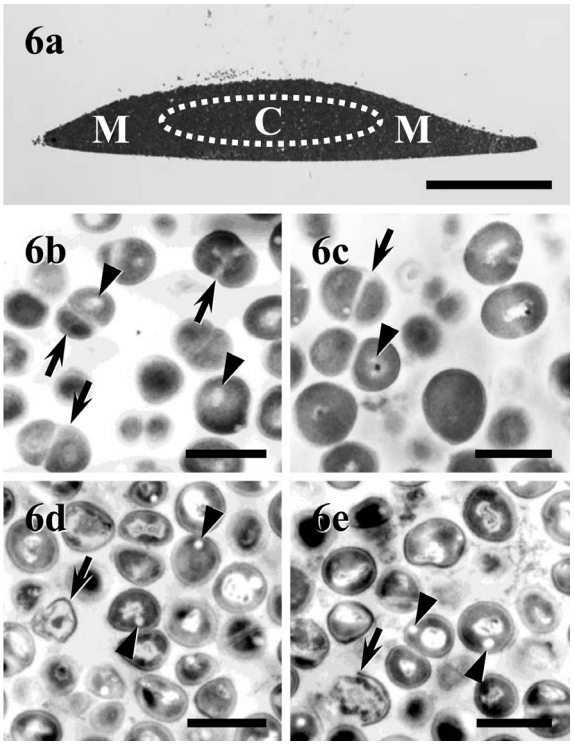

Fig. 6. a) Low-magnification image of bacterial colony of *S. epidermidis*. The colony is vertically sliced at the maximum diameter. M, marginal portion. C, central portion. Bar=50 μm. b, c) Most of *S. epidermidis* cultured for 1 day have a typical structure of bacteria with homogeneous and highly electron-dense cytoplasmic matrix and nucleoid body (arrowheads). Cell-dividing bacteria are plentiful in the marginal portions (b, arrows), but are few in the central portion (c, arrow). d, e) Cell debris (arrows) and vacuolated bacteria (arrowheads) are visible in the central (e) and marginal portions (d) of a bacterial colony cultured for 45 days. Bar=1 μm.

bacteria in the chyme were not increased toward the caudal intestine, even in the rectum.

Degeneration pattern of cultured S. epidermidis: Most of *S. epidermidis* cultured for 1 day possessed cytoplasm divided into two parts: cytoplasmic matrix with homogeneous electron density and nucleoid body with low electron density. Cell division of *S. epidermidis* cultured for 1 day was more frequently found in the marginal portions than in the central portions of bacterial colonies. Cell debris was restricted to the central portions of a bacterial colony cultured for 1 day. Vacuoles were very rarely found in any portions of a bacterial colony cultured for 1 day. In *S. epidermidis* cultured for 45 days, there were few bacteria with intact cytoplasmic matrices, whereas cell debris, such as cell wall remnants, and bacteria possessing vacuoles with low electron density in their cytoplasm were occasionally found in all portions of the colony. Cell debris and vacuolated bacteria were more frequently found in the colony cultured for 45 days than that cultured for 1 day, whereas cell division was less frequent in the colony cultured for 45 days than in that cultured for 1 day (Fig. 6). Lysis or detachment of the cell wall and endospore formations were never found in *S. epidermidis* cultured for either cultivation period.

DISCUSSION

Low-electron-density vacuoles appear in the cytoplasm of *Escherichia coli* cultured in nitrogen-starved culture [8], in that of *Streptoccocus mitis* incubated with glucose [4] and in that of *Nocardia asteroides* grown in a glucose-supplemented medium containing a growth-limiting concentration of nitrogen [10]. Furthermore, treatment with antibiotics, trimethoprim or sulfadiazine, causes cytoplasmic vacuolation in *Enterobacter cloacae*. These vacuoles are also found in *E. cloacae* exposed to edetate disodium (EDTA) plus lysozyme [23] and in *Listeria innocua* exposed to both pulse electric fields and nisin, a representative bacteriocin [5]. On the basis of these findings, vacuolation is considered to be one of degeneration process in bacterial cytoplasm under the unfavorable conditions for bacteria. In the present study, vacuoles were found more in the cytoplasm of *S. epidermidis* cultured for 45 days than in that cultured for 1 day. This result might suggest that these vacuoles occur in the degeneration process progressing over time even without any bactericidal additives. These vacuoles were also found in almost all bacteria having different morphologies that were observed in the present study, and the frequency of indigenous bacteria with vacuoles in the ileal chyme was higher than that in the mucous layer of the ileum and lower than that in the chyme of the large intestine. Furthermore, cell divisions were almost always restricted to indigenous bacteria with less than 10% occupancy by vacuoles. These findings suggested that indigenous bacteria with vacuoles undergo a degeneration process progressing over time regardless of the bacterial strain. This in turn might suggest that the milieu in the intestine was unfavorable for the indigenous bacteria, probably because of the presence of bactericidal substances derived from the host or from the bacteria themselves. The production of bacteria-derived bactericidal substances, such as bacteriocin, could be stimulated by quorum sensing [11, 19, 32]. Because quorum sensing occurs in a manner dependent of bacterial cell density [12], it might occur especially in the large intestine, where densely populated indigenous bacteria possess many vacuoles, as shown in the present study. However, whether or not quorum sensing by indigenous bacteria is involved with their vacuolation in the intestine remains unclear. In the future, the mechanisms underlying vacuolation in indigenous bacteria should be investigated with a focus on the involvement of quorum sensing by indigenous bacteria themselves.

In the rat alimentary tract, bacterial colonies settle on the most luminal portions of the mucosae, which express specific sugars and are speculated to be the origin of indigenous bacteria in the chyme [33, 34]. In the present study, indigenous bacteria with intact cytoplasm were more frequently found in the mucous layer of the ileum than in the ileal chyme, whereas indigenous bacteria with vacuoles were more abundant in the ileal chyme than in the mucous layer of the ileum. From these findings, the sources of indigenous

bacteria in the chyme were ultrastructurally confirmed to be the indigenous bacteria which settle on the most luminal portions of the mucosae. Furthermore, highly vacuolated bacteria were found in the mucous layer and in the chyme in the cecum and ascending colon, but not in the intestinal crypt lumen. From this finding, at least in the cecum and ascending colon, indigenous bacteria are probably provided from the intestinal crypt lumina into the chyme.

In the rat intestine, various bactericidal peptides, such as lysozyme, sPLA2, α-defensin and β-defensin-1 and -2, play important roles in the host defense against indigenous bacteria throughout the digestive tract [27, 35, 36]. Cell wall detachment from the cell membrane is found in rat defensin-treated *Staphylococcus aureus* [28] and also in *E. cloacae* exposed to antibiotics, sulphadiazine or trimethoprim, or to EDTA plus lysozyme [23]. Such detachment is also found in *Pseudomonas fluorescens* and *Acinetobacter* sp. strain MJT/F5/5 exposed to lysozyme [29, 30]. Thornley and Sleytr [30] proposed the mechanisms of lysozyme-mediated detachment of the cell wall in Gram-negative bacteria, as follows: lysozyme removes the peptidoglycan layer between the outer membrane and the cell membrane, so that the outer membrane detaches from the cell membrane. In a previous study, lysozyme is secreted from ileal Paneth cells in response to bacterial hyperproliferation. Furthermore, most of the indigenous bacteria that settle in the large intestine are Gram-negative and strongly immunopositive for lysozyme [35, 36]. In the present study, bacteria with detached cell walls were found throughout the intestine and were more abundant in the mucous layer and chyme in the large intestine than in that in the ileum. On the other hand, bacteria with detached cell walls were never found in *S. epidermidis* cultured with no bactericidal additives, regardless of the incubation period. From these findings, the cell walls of bacteria, probably Gram-negative bacteria, are probably detached from the cell membranes by the effects of bactericidal substances, such as lysozymes. Additionally, in the present study, lysis of the cell wall was also found in indigenous bacteria in both the chyme and mucous layer throughout the intestine, and more frequently found in the chyme of the large intestine than in that of the ileum. Furthermore, dead bacteria were rarely found as cytoplasm remnants without cell walls in the large intestine, but not found at all in the ileum, which might indicate that cell walls could be more quickly lysed than cytoplasm at least in the large intestine. Considering that most of the indigenous bacteria that settled in the large intestine are Gram-negative [35], these findings probably indicate that lysis of the cell wall occurred at least in Gram-negative bacteria. In a previous study, indigenous bacteria were immunopositive for various bactericidal substances, such as lysozyme, sPLA2 and β-defensin-1 and -2 [36], but bactericidal substances for the digestion of the outer membrane of Gram-negative bacteria have not been reported. Therefore, some bactericidal substances or digestive enzymes in the intestine might be able to lyse the cell wall of Gram-negative bacteria as an additional function that differs from their well-known functions. Further investigation is needed to identify the substances that could lyse the cell wall in Gram-negative bacteria.

In general, the number of indigenous bacteria in the chyme increases toward the caudal intestine; 0–10^5 colony forming units (CFU)/m*l* in the stomach to the jejunum, 10^3–10^9 CFU/m*l* in the ileum and 10^{10}–10^{12} CFU/m*l* in the colon of human [25]. From this finding, it is speculated that indigenous bacteria actively proliferate in the large intestine. However, in conflict with this speculation, cell division was less frequently found in the large intestine than in the ileum in the present study. Furthermore, various degenerations, such as vacuolation and lysis and detachment of the cell wall, were more frequently found in the indigenous bacteria in the chyme of the large intestine than in that of the ileum. In spite of these findings, bacterial remnants were not accumulated even in the rectal chyme. The chyme in the rat large intestine is strongly positive for various bactericidal peptides, such as lysozyme, sPLA2 and β-defensin-1 and -2 [36]. From these findings, bacterial remnants derived from indigenous bacteria that were killed in each intestinal segment might be digested probably by the cooperative effects of various bactericidal peptides and digestive enzymes. Enzymes and nutrients from indigenous bacteria have beneficial effects on their hosts [3, 26]. Therefore, the denaturing and killing of large numbers of indigenous bacteria in the intestine might be a host's strategy to utilize bacteria-derived enzymes and nutrients.

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