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—Review—

Ex-Germfree Mice Harboring Intestinal Microbiota Derived from Other Animal Species as an Experimental Model for Ecology and Metabolism of Intestinal Bacteria

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Abstract: *Ex-germfree (GF) animals harboring intestinal microbiota derived from other animal species, e.g. human-flora-associated (HFA) and pig-flora-associated (PFA) mice, have been considered as a tool for studying the ecology and metabolism of intestinal bacteria of man and animals. Human fecal microbiota was transferred into the intestines of the mice with minor modification by inoculating GF mice with human fecal suspensions. Interestingly, bifidobacteria were eliminated from some of the HFA mouse groups, whereas other dominant bacterial groups remained constant. Elimination of bifidobacteria appeared to be dependent on the composition of microbiota in the inoculated sample. Human fecal microbiota established in the intestines of the HFA mice reproduced in the intestine of offspring of these HFA mice and of cage-mated ex-GF mice without any remarkable change in composition. Although the HFA mice could be used for studying the effects of diet on human intestinal microbiota, the metabolism of microbiota of HFA mice reflected that of human feces with respect to some metabolic activities but not others. PFA mice were also a good model for studying the ecosystem of pig fecal microbiota and the control of short chain fatty acids in pig intestines, but not for studying putrefactive products generated in pig intestines. In conclusion, HFA and PFA mice provide a stable and valuable tool for studying the ecosystem and metabolism of the human and animal intestinal microbiota, but they have some limitations as a model.*

Key words: *germfree animal, human-flora-associated mice, intestinal flora, pig-flora-associated mice, putrefactive products*

The endogenous intestinal microbiota interacts closely with the host and the effects of bacteria are both beneficial and harmful to the host [6, 10, 28, 30], but it is difficult to study the effects of intestinal microbiota on humans and domestic animals because of difficulty in standardizing genetic or environmental conditions and the size, and the results of studies on human volunteers and domestic animals are often not clear [2, 8, 29]. In addition, there are ethical problems associated with studies on the influence of intestinal microbiota on

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colonization resistance and effects of toxic chemicals and carcinogenic substances in human volunteers. On the other hand, the intestinal microbiota of experimental animals are quite different from those of humans and domestic animals in composition [19, 26] and in metabolic activities [24]. Therefore, data obtained in animal experiments cannot be directly applied to humans and domestic animals.

To find a solution to this problem, germfree (GF) animals associated with human fecal microbes have been used as a new kind of animal model for studying the intestinal microbiota of humans [3, 7, 13, 22]. By inoculating feces from various species of conventional (CV) animals and humans, the fecal bacteria of the donor animals can be transferred to GF animals [7, 17, 22]. These ex-GF animals harboring the intestinal microbiota derived from other animal species, e.g. human-flora-associated (HFA) and pig-flora-associated (PFA) mice, have been considered as a tool for studying the ecology and metabolism of intestinal bacteria of human and domestic animals [4, 5, 25, 31].

Production of HFA mice

GF BALB/c mice were inoculated with the fecal suspension of two healthy human adults and the development and stability of the intestinal microbiota in the HFA mice were examined [13]. Although the number of enterobacteriaceae in the developing microbiota was high compared with those in the feces used in the inoculum for the first two days, they decreased rapidly from the third day (Figs. 1 and 2). Conversely, anaerobic bacteria were present in low concentrations on day 1 and increased thereafter. On day 3, anaerobic bacteria which were dominant in the human feces became dominant in the HFA mice. Interestingly, bifidobacteria were eliminated from HFA mice inoculated with feces from one of the donors within 2–8 weeks, but other dominant bacterial groups remained constant (Fig. 2).

The bacterial composition of the microbiota in the feces of HFA mouse groups inoculated with fecal suspensions from six different volunteers was examined and compared [11]. The numbers of eubacteria, peptococcaceae and enterobacteriaceae in HFA mouse groups were similar to those in humans (Fig. 3). The number of these bacterial groups was significantly greater than those in CV mice and the numbers of total bacteria, bacteroidaceae, clostridia and streptococci were greater than those in humans and CV mice [11]. The variations in the composition of the fecal microbiota among HFA mouse groups were not so obvious, and individual variations among the inoculated human fe-

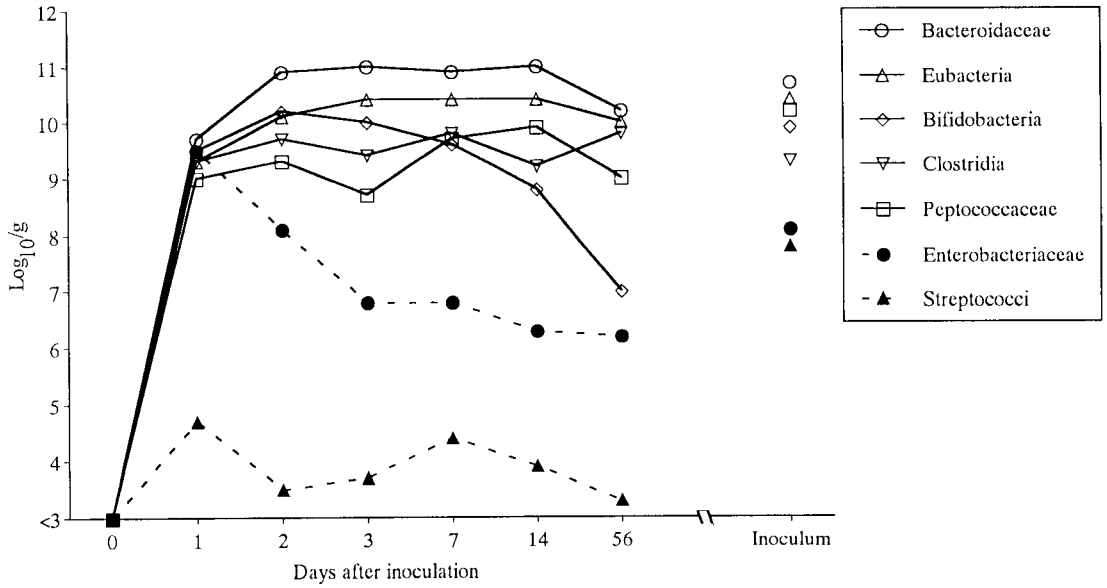

Fig. 1. Composition of major bacterial components of fecal microbiota of HFA mice after inoculation of human fecal suspension A.

ces were not reflected in the HFA mouse groups. Bifidobacteria were also eliminated from three out of six HFA mouse groups in this study. The elimination of bifidobacteria seemed to be dependent on the composition of microbiota in the inoculated sample.

Transfer of intestinal microbiota of HFA mice

Development of intestinal microbiota in newborn offspring of HFA mice was investigated [15]. Facultative anaerobes were detected from the first day after birth,

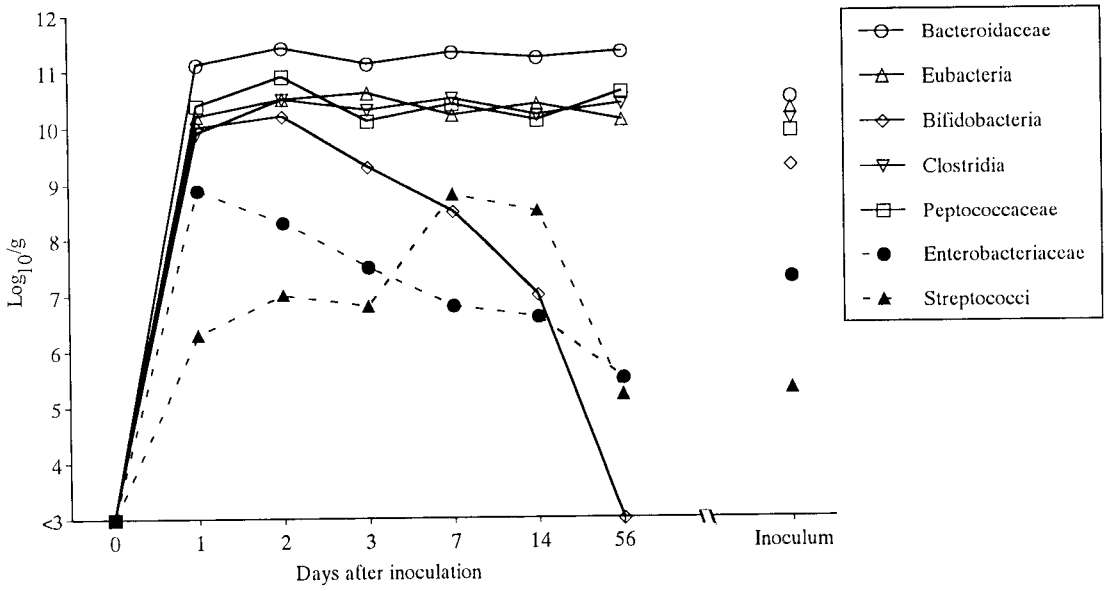

Fig. 2. Composition of major bacterial components of fecal microbiota of HFA mice after inoculation of human fecal suspension B.

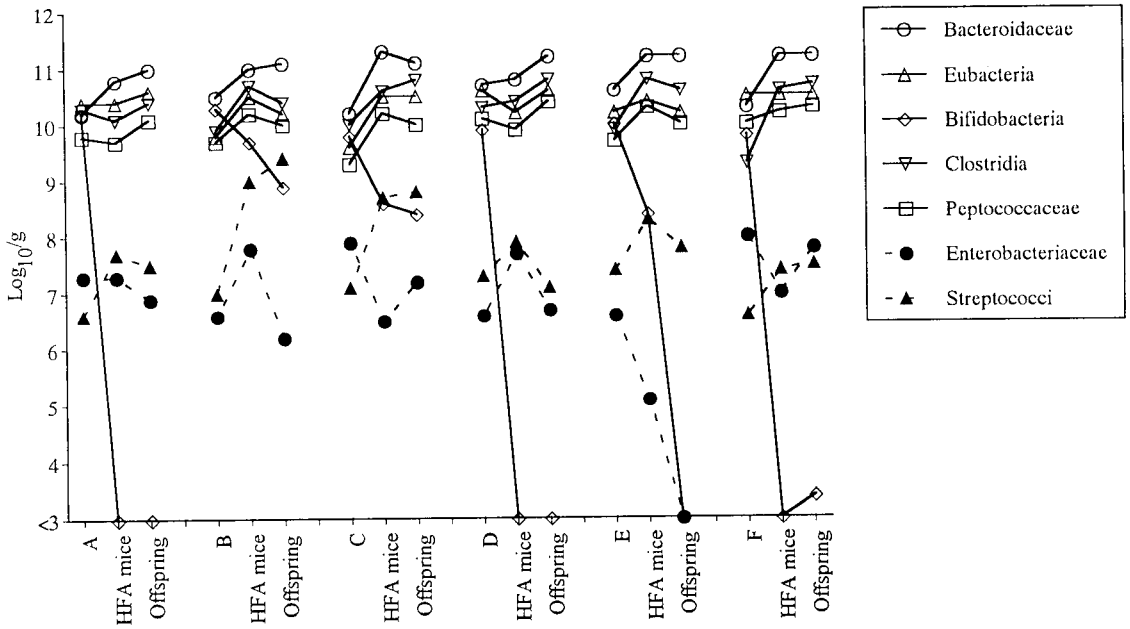

Fig. 3. Composition of major bacterial components of fecal microbiota of humans (A-F), HFA mice and offspring of HFA mice.

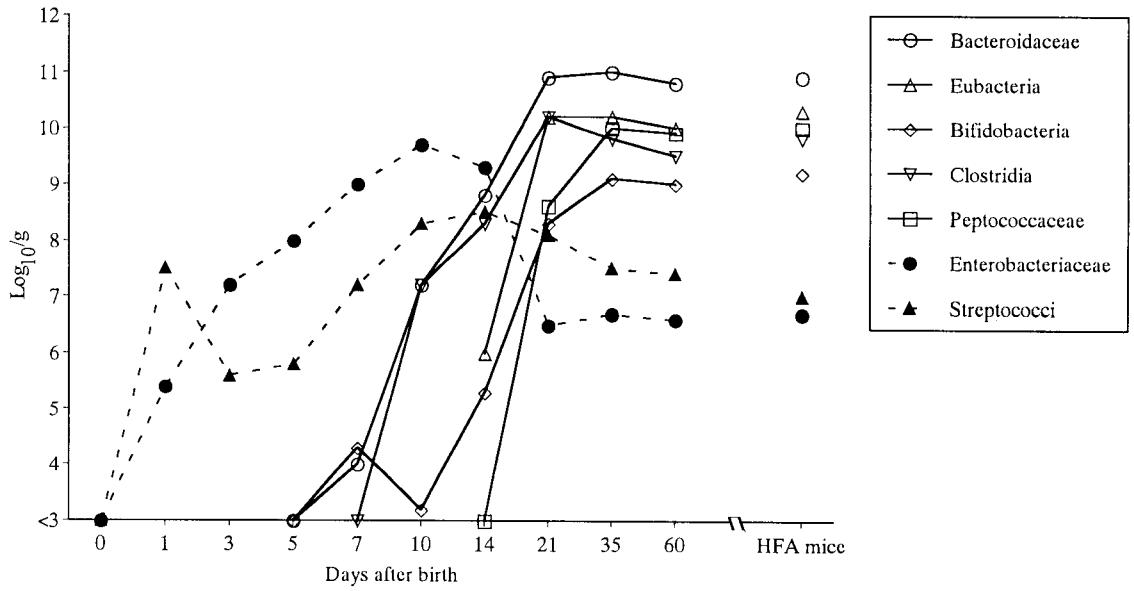

Fig. 4. Development of intestinal microbiota of offspring of HFA mice.

but anaerobes were not detected in the first week (Fig. 4). Anaerobes rapidly increased from the 2nd week after birth and became predominant in the newborn intestines. Most of the intestinal bacteria of mother HFA mice colonized in the intestines of offspring of HFA mice within 3 weeks after birth. The compositions of offspring of HFA mice were similar to those of their mothers. Fecal bacterial composition of offspring of HFA mice inoculated with 6 different human feces was also similar to that of their mothers (Fig. 3). These findings indicate that the human intestinal microbiota established in the intestine of HFA mice reproduced in the intestine of the offspring of the HFA mice without any remarkable change in composition and could be maintained for a long period by breeding.

On the other hand, the development of intestinal microbiota in the offspring of HFA mice was similar to that in CV mice [15] but not to that in human infants. In human infants, bifidobacteria become the most predominant bacteria within 1 week after birth [1, 20], but bifidobacteria never became the most predominant bacteria in the infant offspring of HFA mice, even though the mother HFA mice harbored a large population of bifidobacteria (Fig. 4). These findings indicated that the offspring of HFA mice cannot simulate the development of intestinal microbiota of the human infant.

The intestinal microbiota of HFA mice were also reproduced by cage-mating. HFA mice were cage-mated with GF mice and the composition of intestinal microbiota of the ex-GF mice was examined [14]. The numbers of aerobes rapidly increased and they became the predominant bacterial group on the first day after cage-mating, whereas anaerobes were detected in relatively low numbers (Fig. 5). In a few days after exposure to bacteria of donor HFA mice, anaerobes increased and became the predominant bacteria but the numbers of aerobes decreased. The composition of intestinal microbiota of ex-GF mice became similar to that of HFA mice by 8 days after contact with HFA mice, and this balance was maintained thereafter.

Metabolic activities of intestinal microbiota of HFA mice

Mallett *et al.* [18] demonstrated that certain enzymic activities of human fecal microbiota can be simulated in rats associated with human intestinal bacteria. Hirayama *et al.* [11] reported that the activities of β-glucosidase and β-glucuronidase in HFA mice were similar to those in humans and different from those in CV mice (Fig. 6), but nitroreductase activity of HFA mice was intermediate between those of humans and CV mice, and nitrate reductase of HFA mice showed higher activity than those of humans and CV mice. Although variations in en-

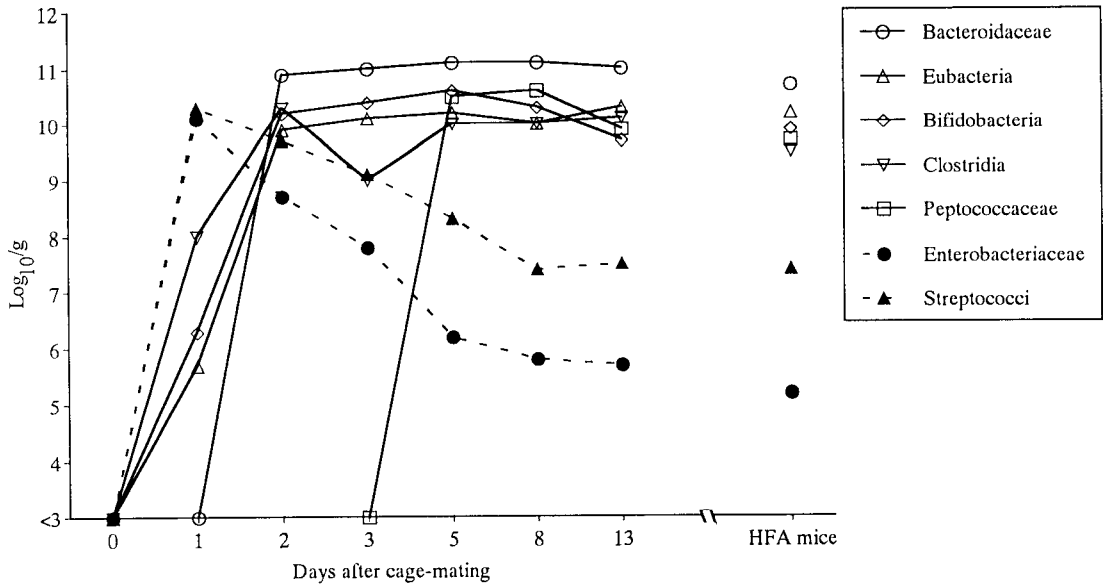

Fig. 5. Change in fecal microbiota of ex-GF mice cage-mated with HFA mice.

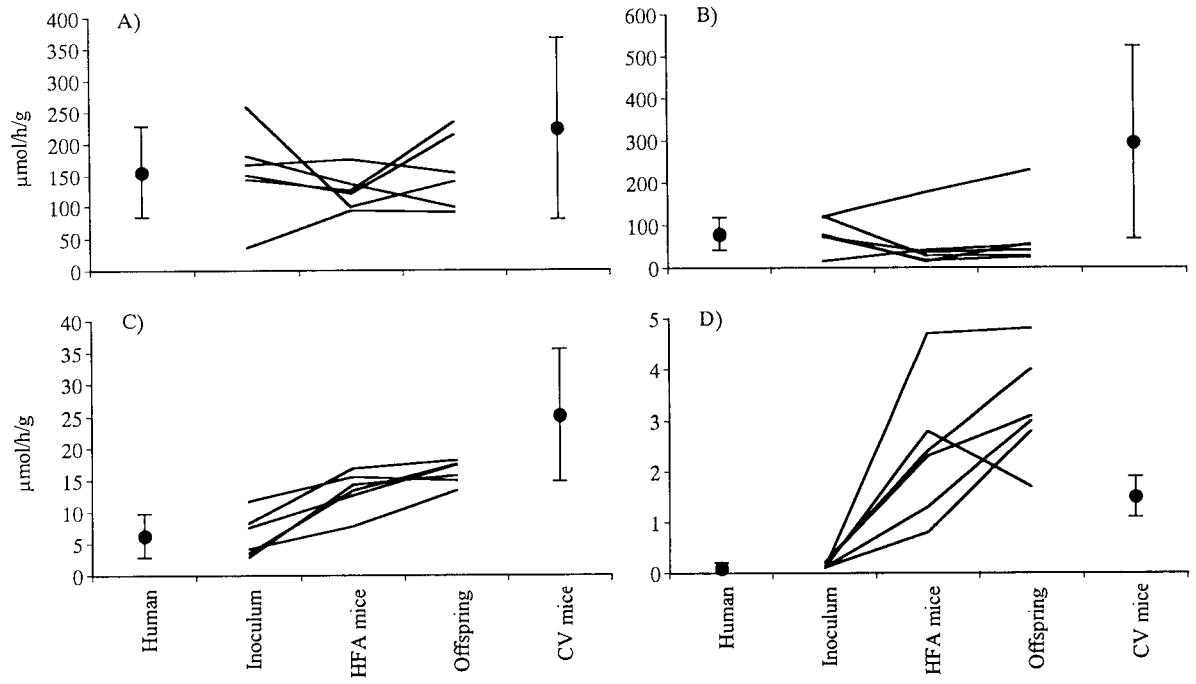

Fig. 6. Enzyme activities in feces of humans, HFA mice, offspring of HFA mice and CV mice. A) β-glucosidase, B) β-glucuronidase, C) nitroreductase, D) nitrate reductase.

zyme activities were observed among both HFA mouse groups and inoculated human feces, the variations among HFA mouse groups did not correspond to those among human feces. The activities of fecal enzymes of HFA mice were similar to those of their offspring.

The concentrations of putrefactive products in the feces of HFA mice were much lower than those in human feces and similar to those in CV mice (Fig. 7),

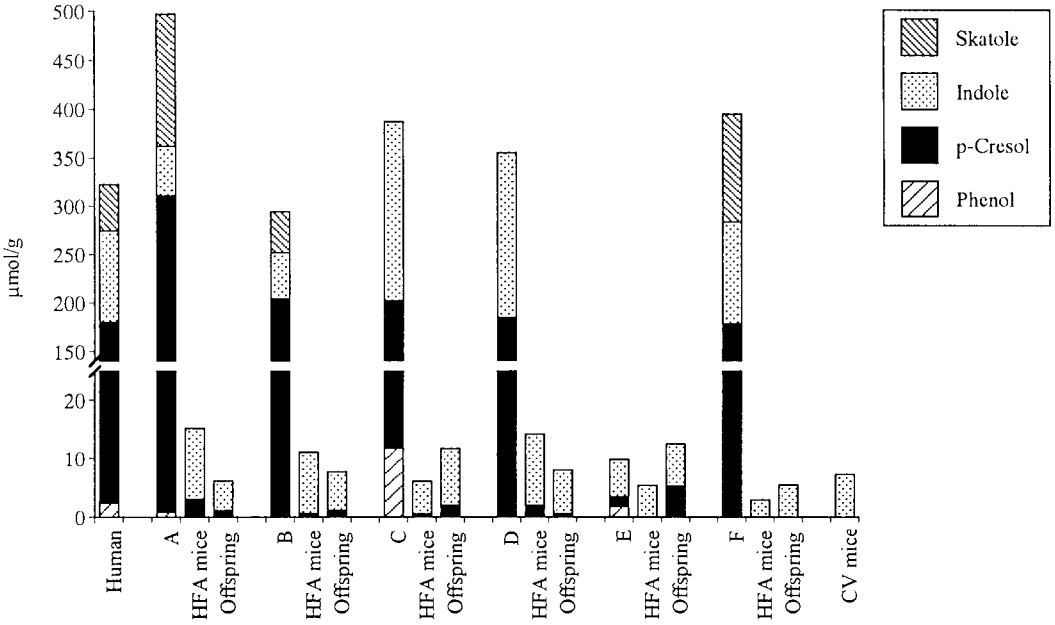

Fig. 7. Concentration of putrefactive products in feces of humans (A–F), HFA mice, offspring of HFA mice and CV mice.

but *p*-cresol was detected in most HFA mouse feces but not in CV mouse feces, and the concentration of fecal indole in some of the HFA mouse groups was significantly higher than that in CV mice. In spite of the remarkable variations among individuals observed in the concentration and composition of human fecal putrefactive products, there were only slight variations among HFA mouse groups.

Although the concentration of fecal short chain fatty acids (SCFAs) in HFA mice was significantly lower than that in humans and similar to that in CV mice, the composition of SCFAs in HFA mice was closer to that in humans than that in CV mice (Fig. 8).

Bacterial metabolism in the intestine of HFA mice therefore reflected that of human feces with respect to some metabolic activities but not others, even though the bacterial composition of the feces of HFA mice was similar to that of the inocula [11].

Application of HFA mice to studies on the effects of dietary supplements on intestinal microbiota of humans

Although some reports showed significant changes in fecal microbiota with a high meat diet [23], others found the effects of dietary supplementation of bran or beef protein in human volunteers were small [2, 8]. In the study by Hirayama *et al.* [14], HFA mice were fed a high-meat (HM) diet and high-bran (HB) diet to investigate the effects of diet on the intestinal microbiota. The number of enterobacteriaceae significantly increased with the HM diet, and decreased with the HB diet to the level of the basal diet. The numbers of bacteroidaceae, clostridia and streptococci decreased with the HB diet, whereas the number of bifidobacteria in HFA mice fed the HB diet was significantly higher than in those fed the HM or basal diet.

Fructooligosaccharides (FOS) are dietary fiber-like saccharides which improve intestinal microbiota with proliferation of bifidobacteria [9]. FOS were administered to HFA mice in drinking water at a concentration of 0.1% and 0.5% and the composition of fecal microbiota was examined [14]. The ratio of bifidobacteria to total bacteria increased and the number of bacteroidaceae and enterobacteriaceae tended to decrease in HFA mice administered FOS solution, although the differences were not statistically significant.

These studies suggest that the influences of different dietary components may be detected more clearly in

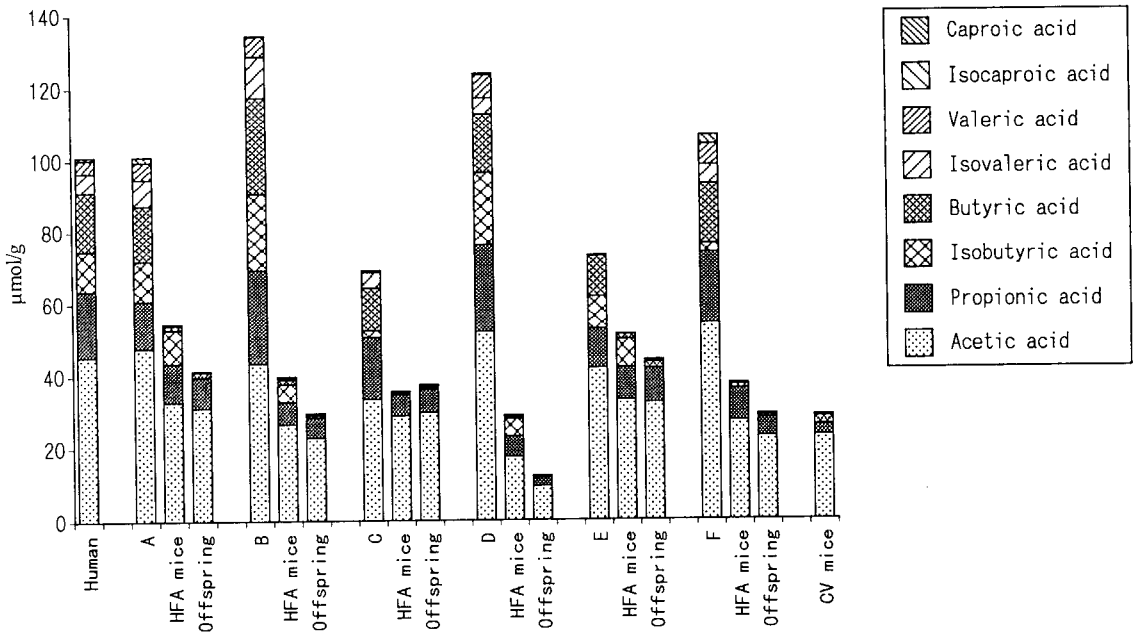

Fig. 8. Concentration of short chain fatty acids in feces of humans (A–F), HFA mice, offspring of HFA mice and CV mice.

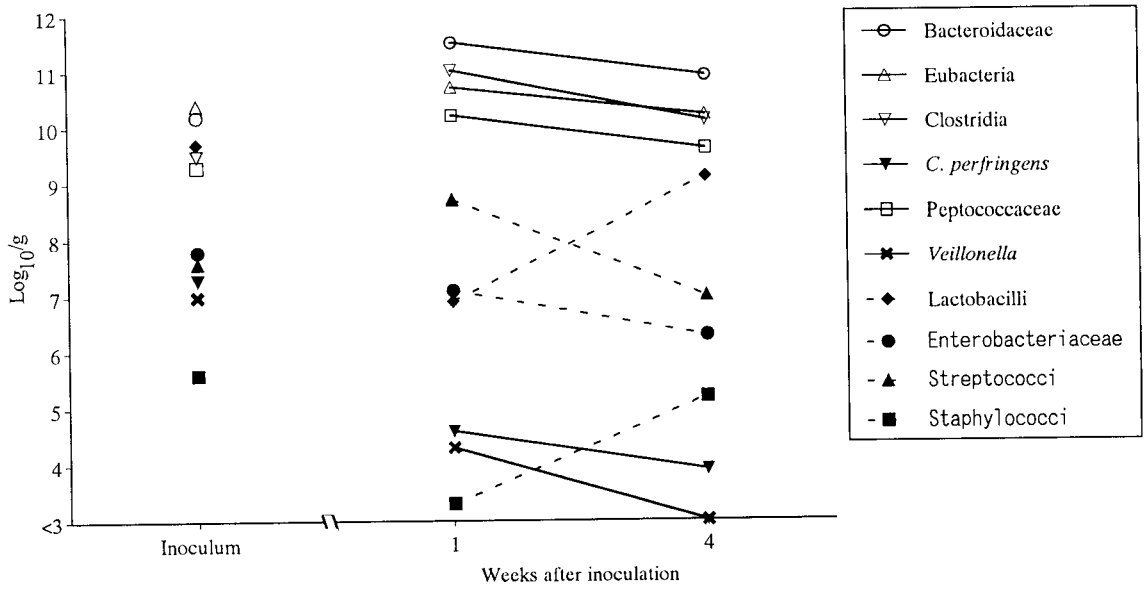

Fig. 9. Composition of major bacterial components of fecal microbiota of PFA mice inoculated with fecal suspension of a 20-day-old piglet.

HFA mice than in humans and demonstrated that HFA mice are useful for studying the effects of diet on human intestinal microbiota.

Production of PFA mice

Although Ducluzeau *et al.* [4] reported that mice inoculated with fecal microbiota of adult pigs or piglets serve as a tool for studying porcine gastrointestinal

microbiota, there are few reports on the composition and metabolic activities of intestinal bacteria of mice inoculated with the feces of pigs. Hirayama *et al.* [12] produced PFA mice by inoculating a fecal suspension of pigs before and after weaning, and examined the bacterial composition of the fecal microbiota and the concentrations of SCFAs and putrefactive metabolites in the cecal contents of PFA mice. Major fecal bacteria of pigs colonized in the intestines of PFA mice, and the composition of microbiota in the PFA mouse feces was similar to that of the pig fecal inoculum (Fig. 9). Surprisingly, lactobacilli were detected in the feces of PFA mice even 4 weeks after inoculation with pig feces at levels of 10^6–10^9/g feces. It has been reported that lactobacilli have strong host-specificity [16, 21, 32], and that they do not colonize in the intestines of mice inoculated with feces from pigs, piglets [4] or humans [5]. Since lactobacilli are one of the most predominant bacteria in the intestines of pigs both before and after weaning, PFA mice which harbor lactobacilli of pig origin should be a valuable tool for studying the ecology of pig intestinal microbiota.

On the other hand, fecal concentrations of putrefactive products in PFA mice did not simulate those in the inocula, although the concentrations of the major components of SCFAs of PFA mice were similar to those of the inocula. These findings indicate that PFA mice are a good model for studying the ecosystem of pig fecal microbiota and control of SCFAs in pig intestines, but not for studying putrefactive products generated in pig intestines.

Conclusion

Ex-GF animals harboring intestinal microbiota originating in other animal species, e.g. HFA and PFA mice, provide a stable and valuable tool for studying the ecosystem and metabolism of human fecal microbiota, but they have some limitations as a model. Further studies concerning the composition of intestinal bacteria of HFA and PFA mice at the species level, and differences in intestinal physiological conditions among human, domestic animals and mice, are needed. In addition, Ducluzeau *et al.* [4] showed that the composition of diet greatly influences the composition of intestinal bacteria of PFA mice. Rumney *et al.* [27] reported that the metabolic activities of intestinal bacteria of HFA rats appeared to be dependent on diet, and the results obtained with HFA rats were particularly relevant to humans when the animals were fed a human diet. Development of a special diet for HFA and PFA mice is required to establish a better model to study the metabolism of human and pig intestinal microbiota.

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References

1. Benno, Y. and Mitsuoka, T. 1986. Development of intestinal microflora in human and animals. *Bifidobacteria Microflora* 5: 13–25.
2. Drasar, B.S., Jenkins, D.J.A., and Cummings, J.H. 1976. The influence of a diet rich in wheat fibre on the human faecal flora. *J. Med. Microbiol.* 9: 423–431.
3. Ducluzeau, R., Ladiré, M., and Raibaud, P. 1984. Effect of bran ingestion on the microbial faecal floras of human donors and of recipient gnotobiotic mice, and on the barrier effects exerted by these floras against various potentially pathogenic microbial strains. *Ann. Microbiol.* 135A: 303–318.
4. Ducluzeau, R., Rapine, C., Courvalin, C., and Raibaud, P. 1978. Transfer of the fecal microbial flora from holoxenic piglets and adult pigs to axenic piglets and axenic adult mice: effect of the animal host and of the diet on the fecal microbial pattern of these animals. *Ann. Microbiol.* 129B: 597–612.
5. Fujiwara, S., Hirota, T., Nakazato, H., Mizutani, T., and Mitsuoka, T. 1991. Effect of konjac mannan on intestinal microbial metabolism in mice bearing human flora and in conventional F344 rats. *Food Chem. Toxicol.* 29: 601–606.
6. Gordon, H.A. and Pesti, L. 1971. The gnotobiotic animal as a tool in the study of host microbial relationships. *Bacteriol. Rev.* 35: 390–429.
7. Hazenberg, M.P., Bakker, M., and Verschoor-Burggraaf, A. 1981. Effects of the human intestinal flora on germ-free mice. *J. Appl. Bacteriol.* 50: 95–106.
8. Hentges, D.J., Maier, B.R., Burton, G.C., Flymm, M.A., and Tsutakawa, R.K. 1977. Effect of a high-beef diet on the fecal bacterial flora of humans. *Cancer Res.* 37: 568–571.
9. Hidaka, H., Tashiro, Y., and Eida, T. 1991. Proliferation of bifidobacteria by oligosaccharides and useful effect on human health. *Bifidobacteria Microflora* 10: 65–79.
10. Hill, M. 1988. Gut flora and cancer in humans and laboratory animals. pp. 461–502. *In*: Role of the Gut

Microflora in Toxicity and Cancer (Rowland, I.R. ed.), Academic Press, London.
11. Hirayama, K., Itoh, K., Takahashi, E., and Mitsuoka, T. 1995. Comparison of composition of faecal microbiota and metabolism of faecal bacteria among human-flora-associated (HFA) mice inoculated with six different human faeces. *Microbial Ecol. Health Dis.* 8: 199–211.
12. Hirayama, K., Itoh, K., Takahashi, E., Shinozaki, K., and Sawasaki, T. 1996. Composition of faecal microbiota and metabolism of faecal bacteria of pig-flora-associated (PFA) mice. *Microbial Ecol. Health Dis.* 9: 199–206.
13. Hirayama, K., Kawamura, S., and Mitsuoka, T. 1991. Development and stability of human faecal flora in the intestine of ex-germ-free mice. *Microbial Ecol. Health Dis.* 4: 95–99.
14. Hirayama, K., Mishima, M., Kawamura, S., Itoh, K., Takahashi, E., and Mitsuoka, T. 1994. Effects of dietary supplements on the composition of fecal flora of human-flora-associated (HFA) mice. *Bifidobacteria Microflora* 13: 1–7.
15. Hirayama, K., Miyaji, K., Kawamura, S., Itoh, K., Takahashi, E., and Mitsuoka, T. 1995. Development of intestinal flora of human-floral-associated (HFA) mice in the intestine of their offspring. *Exp. Anim.* 44: 219–222.
16. Lin, J.H. and Savage, D.C. 1984. Host specificity of the colonization of murine gastric epithelium by lactobacilli. *FEMS Microbiol. Lett.* 24: 67–71.
17. Maejima, K., Sasaki, J., Shimoda, K., and Kurosawa, T. 1981. Bacterial flora of ex-germfree mice after oral inoculation of feces from various species of conventional animals. *Exp. Anim.* 30: 157–160.
18. Mallett, A.K., Bearne, C.A., Rowland, I.R., Farthing, M.J.G., Cole, C.B., and Fuller, R. 1987. The use of rats associated with a human faecal flora as a model for studying the effects of diet on the human gut microflora. *J. Appl. Bacteriol.* 63: 39–45.
19. Mitsuoka, T. 1982. Recent trends in research on intestinal flora. *Bifidobacteria Microflora* 1: 3–24.
20. Mitsuoka, T. and Hayakawa, K. 1972. Die Darmflora bei Menschen. I. Mitteilung. Die Zusammensetzung der Faekalflora der verschiedenen Altergruppen. *Zbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. I. Orig. A* 233: 333–352.
21. Morishita, Y., Mitsuoka, T., Kaneuchi, C., Yamamoto, S., and Ogawa, M. 1971. Specific establishment of lactobacilli in the digestive tract of germ-free chickens. *Jpn. J. Microbiol.* 15: 531–538.
22. Raibaud, P., Ducluzeau, R., Dubos, F., Hudault, S., Bewa, H., and Muller, M.C. 1980. Implantation of bacteria from the digestive tract of man and various animals into gnotobiotic mice. *Am. J. Clin. Nutr.* 33: 2440–2447.
23. Reddy, B.S., Weisburger, J.H., and Wynder, E.L. 1975. Effects of high risk and low risk diets for colon carcinogenesis on fecal microflora and steroids in man. *J. Nutr.* 105: 878–884.
24. Rowland, I.R., Mallett, A.K., and Bearne, C.A. 1986. Enzyme activities of the hindgut microflora of laboratory animals and man. *Xenobiotica* 16: 519–523.
25 Rowland, I.R. and Tanaka, R. 1993. The effects of transgalactosylated oligosaccharides on gut flora metabolism in rats associated with a human faecal microflora. *J. Appl. Bacteriol.* 74: 667–674.
26. Rumney, C.J. and Rowland, I.R. 1992. *In vivo* and *in vitro* models of the human colonic flora. *Crit. Rev. Food Sci. Nutr.* 31: 299–331.
27 Rumney, C.J., Rowland, I.R., and O'Neill, I.K. 1993. Conversion of IQ to 7-OHIQ by gut microflora. *Nutr. Cancer* 19: 67–76.
28. Simon, G.L. and Gorbach, S.L. 1984. Intestinal flora in health and disease. *Gastroenterol.* 86: 174–193.
29. Sissons, J.W. 1989. Potential of probiotic organisms to prevent diarrhoea and promote digestion in farm animals—A review. *J. Sci. Food Agric.* 49: 1–13.
30. Tancrède, C. 1992. Role of human microflora in health and disease. *Eur. J. Clin. Microbiol. Infect. Dis.* 11: 1012–1015.
31. Tancrède, C., Andremont, A., and Kherbiche, A.M. 1981. Germfree mice associated with human flora as a means of bacteriological surveillance of neutropenic patients in sterile environments. pp. 755–758. *In*: Recent Advances in Germfree Research (Sasaki, S., Ozawa, A., Hashimoto, K. eds.), Tokai University Press, Tokyo.
32. Tannock, G.W., Szylit, O., Duval, Y., and Raibaud, P. 1982. Colonization of tissue surfaces in the gastrointestinal tract of gnotobiotic animals by lactobacillus strains. *Can. J. Microbiol.* 28: 1196–1198.