

The Variation and Adherence of the Species of *Bifidobacterium* in the Intestine during Oral Administration of *Bifidobacterium*

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Studies on *Bifidobacterium* have been conducted for a long time, but in recent years preparations containing *Bifidobacterium* have clinically been used extensively, and regarded to be useful for improvement in digestive function in particular, normalization of a bacterial flora in the intestinal tract, etc. We have also previously reported this problem^{1,2)}, but in the present study we examined the colonization and proliferation of *Bifidobacterium* administered in the intestinal tract.

SUBJECTS AND METHODS

Patients with chronic hematological diseases, not using antimicrobial antibiotics, were selected as clinical cases. Normal mice (C3H/He) were used in animal experiments. In both subjects, we examined variations in the species of *Bifidobacterium* (residential *Bifidobacterium* and administered *Bifidobacterium*) in feces before, during, and after administration.

As for the administered *Bifidobacterium*, we used a preparation which had already been reported^{1,2)}. Namely, we used cow's milk containing about 10^7 /ml of *Bifidobacterium longum* (*B. longum*) and about 10^7 /ml of *Lactobacillus acidophilus* (*L. acidophilus*) (Morinaga Bifidus[®], made by Morinaga Milk Industry Co., Ltd.).

Daily, 200 ml of this milk was administered in clinical cases, while about 10 ml was administered daily to mice. (No water was administered and a daily exchange was made with fresh cow's milk containing *Bifidobacterium*. In addition, solid feed was concomitantly given.)

The isolation, identification, and assay of *Bifidobacterium* were conducted in accordance with the previous report. The isolation medium was a BS medium³⁾ and the assay was made by the 10-fold dilution method. In detail, concerning the bacterial species, a specimen was diluted stepwise by the 10-fold

dilution method, the colonies of the final dilution showing bacterial growth on a medium were grouped morphologically, one colony from each group was selected for confirmation of the bacterial species, and the bacterial counts of *Bifidobacterium* was calculated from the colony number. (Even an appearance of morphologically different colony may result in the same species.) When the colony number was small, the assay was carried out on the colonies on a medium diluted one step previously. Even if the examined colony was the same species, it is difficult to say that the whole of colony was the same species but the majority may be considered to be identical. For confirmation of the species, carbohydrate fermentation and other biochemical tests were performed, and the species was determined in accordance with the classification of Bergy⁴⁾. The bacterial quantity was expressed as the bacterial count per 1 g of feces.

RESULTS

Table 1 shows variation in *Bifidobacterium* species in clinical cases (3 cases) during *B. longum* (Morinaga Bifidus[®]) administration. In all three cases, residential *Bifidobacterium* was mainly *Bifidobacterium adolescentis* (*B. adolescentis*), but 3 months later naturally *B. longum* increased in feces in all three cases, furthermore an increase in *B. adolescentis* was observed. In Case 1, both species increased continuously for 9 months during *B. longum* administration, and the administered *Bifidobacterium* was predominant over the residential *Bifidobacterium* after 9 months. In Case 2, too, *Bifidobacterium* increased up to 6 months, and it was observed that both species reached the identical count after 6 months. Also, at 1 month after termination of administration, both the residential and administered bifidobacteria were decreased, however they kept higher bacterial counts

Table 1 Variation in *Bifidobacterium* species in feces during oral administration of *B. longum* to man (*B. adolescentis*: residential bacteria, *B. longum*: orally administered bacteria)

(Figures show bacterial count in 1 g of feces.)

	Species	Before	During administration of <i>B. longum</i>			After administration (1 month)
			3 months	6 months	9 months	
Case 1	<i>B. adolescentis</i> <i>B. longum</i>	4×10 ⁷ 0	9×10 ⁷ 3×10 ⁷	6×10 ⁸ 2×10 ⁸	1×10 ⁹ 3×10 ⁹	
Case 2	<i>B. adolescentis</i> <i>B. longum</i>	1×10 ⁸ 0	9×10 ⁸ 3×10 ⁸	2×10 ¹⁰ 2×10 ¹⁰	ended after 6 month	
Case 3	<i>B. adolescentis</i> <i>B. longum</i>	1×10 ⁸ 0	3×10 ⁸ 1×10 ⁸	administration ended after 3 months		3×10 ⁸ 0

Table 2 Variation in *Bifidobacterium* species in feces during oral administration of *B. longum* to mice (*B. pseudolongum*: residential bacteria, *B. longum*: orally administered bacteria)

(Figures show bacterial count in 1 g of feces.)

	Species	Before	During administration of <i>B. longum</i>			After administration (1 month)
			2 months	4 months	6 months	
Mouse (mean of 5 mice)	<i>B. pseudolongum</i> <i>B. longum</i>	3×10 ⁷ 0	3×10 ¹⁰ 1×10 ¹⁰	6×10 ¹⁰ 2×10 ¹⁰	3×10 ¹¹ 1×10 ¹¹	5×10 ¹⁰ 0

than those before administration. In Case 3, too, both bifidobacterial species showed an increase in 3 months, but administration was discontinued after 3 months. The residential bacteria without a change in the quantity remained in feces 1 month after discontinuance, but the administered bifidobacteria were not observed and no colonization was observed.

Similar examinations were carried out in 5 mice, and those results are shown in Table 2. The residential bifidobacteria chiefly consisted of *Bifidobacterium pseudolongum* (*B. pseudolongum*) but both increases in the residential and administered bifidobacteria were observed as the administration month number increased, similarly to the clinical cases. However, no increase in the administered bifidobacteria more than the residential bifidobacteria was observed, unlike the clinical cases. Moreover, no administered bifidobacteria was observed 1 month after the termination of administration.

DISCUSSION

As the clinical application of *Bifidobacterium* preparations, it has works of normalizing digestive function and in nutritional aspects as mentioned in the past, but we observed that there was an imbalance in the normal intestinal flora in immunodeficiency, particularly during administration of immunosuppressive, anticancer, or antileukemic drugs, and men-

tioned⁹⁾ that *Bifidobacterium* administration was effective in normalization of intestinal flora on such an occasion. Also, we reported⁹⁾ that this *Bifidobacterium* administration was helpful for inhibition of *Candida* proliferation in the intestinal tract and for prevention of secondary infections.

In the present study, *Bifidobacterium* in feces was examined as to whether administered *Bifidobacterium* was colonized and proliferating in case *Bifidobacterium* was orally administered. There are several kinds of bifidobacterial species constantly living in the human intestinal tract, and there are individual differences, varying in individuals; some have only one species and some have several species. Mitsuoka also reported that there were differences between adults and infants and also that adults had several species of *Bifidobacterium*⁷⁾. In the present study of clinical cases, milk containing *B. longum* was administered to patients mainly having *B. adolescentis* as a kind of *Bifidobacterium*, and the presence or absence of *B. longum* colonization was examined. As a result, an increase in *B. longum* was observed following long-term administration of *B. longum* and moreover it was observed that the administered bifidobacteria increased more than the residential bifidobacteria in some cases. Colonization and proliferation were observed in some cases 1 month after termination of administration, but in contrast no colonization of the administered bacteria was observed in some cases.

In mice (mainly only *B. pseudolongum* as the residential *Bifidobacterium*), too, similarly to man, needless to say the administered bacteria, *B. pseudolongum*, as the residential bacteria, increased following *B. longum* administration, which did not increase more than the residential bacteria, also no administered bacteria were observed 1 month after termination of administration, and no colonization or proliferation of the administered bacteria was observed. This seems attributable to the difficulty of colonization because the administered *B. longum* is not the residential *Bifidobacterium* in mice.

There is a report by Honma, et al.⁸⁾ that *Bifidobacterium* administered from the outside is possible to colonize, concerning the problem of colonization and proliferation, while Ichihashi⁹⁾ states that there are many factors involved in the formation, variation, difference, etc. of the colonization of intestinal bacteria in man. In the present study, too, considerable differences were observed, depending on the case in man, and accordingly it seems necessary to examine cases of long-term discontinuance after long-term administration henceforth.

Also, in both human and animal experiments, an increase in the residential *Bifidobacterium* was observed following *B. longum* administration, but this seems attributable to the results that the essential conditions for the growth of *Bifidobacterium* such as the pH change in the intestinal tract and others were satisfied by *Bifidobacterium* administration. Moreover, *L. acidophilus* contained in milk naturally increased, but this result had already been reported^{1,2)} and was omitted.

For *Bifidobacterium* to act strongly and more effectively in the live body, the colonization and proliferation of the administered *Bifidobacterium* may be needed. For this purpose, it is necessary to prepare the conditions in which *Bifidobacterium* is most likely to grow in the intestinal tract. With respect to the conditions in the intestinal tract, it may be necessary to consider the pH in the intestinal tract, secretion of bile, correlation with other predominant intestinal bacteria, and presence of growth factors of *Bifidobacterium*, but also the immunity conditions in the intestinal tract, etc. as the conditions on the live body side. As mentioned already in the present report, the colonization and proliferation of *Bifidobacterium* showed different characters in respective cases, and this seems attributable to involvement of many factors. It is considered that the effect of *Bifidobacterium* administration shows even increased results by administering *Bifidobacterium* with constant consideration of these factors.

CONCLUSION

B. longum was orally administered to men and mice, and variations in bifidobacterial species in feces was examined.

In man both *B. longum* and *B. adolescentis*, the residential *Bifidobacterium*, increased following *B. longum* administration. The administered *Bifidobacterium* increased more than the residential *Bifidobacterium* in some cases. The administered *B. longum* colonized and proliferated 1 month after the termination of *B. longum* administration in some cases, but disappeared in others.

In mice, too, both *B. longum* and *B. pseudolongum*, the latter of which is the residential *Bifidobacterium*, increased following *B. longum* administration, but the administered *Bifidobacterium* showed no increase beyond the constant *Bifidobacterium*, and the administered *B. longum*, disappeared 1 month after the termination of administration.

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