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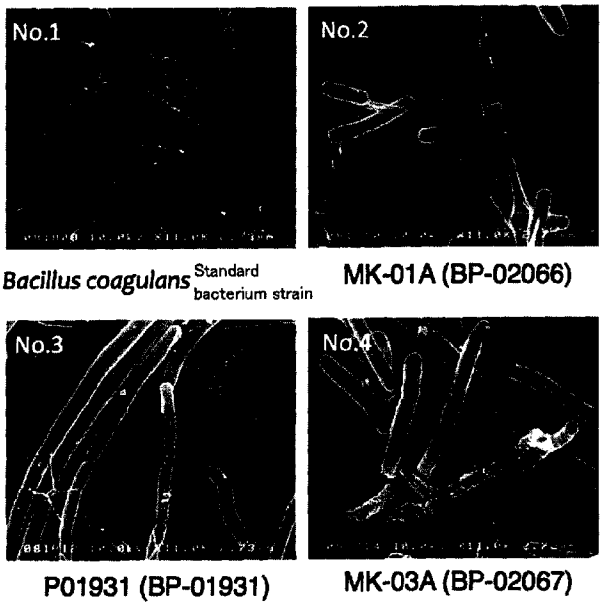

(57) Abrégé/Abstract:
The diatheses of animals vary depending on genetic backgrounds of the animals, and the microbial structure of an enterobacterial flora inherent in a host and the behavior of the concentration of a biological molecule sometimes vary depending on the diathesis of

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the host. In this case, it is needed to administer a proper probiotic.
Provided is a microbial preparation for controlling the proportion of the population of bacteria belonging to the division Bacteroidetes, Firmicutes or Proteobacteria or the proportion of the population of bacteria belonging to the genus Clostridium, Lactobacillus, Bifidobacterium or Bacteroidetes in the enterobacterial florae in an animal body, and for controlling the concentration of a functional molecule contained in a living body, said microbial preparation containing a microorganism P01931 (International Accession No. BP-1931), MK-01A (International Accession No. BP-02066) or MK-03A (International Accession No. BP-02067) or a component of the microorganism.

ABSTRACT

The diatheses of animals vary depending on genetic backgrounds of the animals, and the microbial structure of an enterobacterial flora inherent in a host and the behavior of the concentration of a biological molecule sometimes vary depending on the diathesis of the host. In this case, it is needed to administer a proper probiotic.

Provided is a microbial preparation for controlling the proportion of the population of bacteria belonging to the division Bacteroidetes, Firmicutes or Proteobacteria or the proportion of the population of bacteria belonging to the genus Clostridium, Lactobacillus, Bifidobacterium or Bacteroidetes in the enterobacterial florae in an animal body, and for controlling the concentration of a functional molecule contained in a living body, said microbial preparation containing a microorganism P01931 (International Accession No. BP-1931), MK-01A (International Accession No. BP-02066) or MK-03A (International Accession No. BP-02067) or a component of the microorganism.

PROBIOTIC OR PREBIOTIC, METHOD FOR PRODUCING SAME, MICROBIAL PREPARATION, HEALTH FOOD, AND MEDICINE

TECHNICAL FIELD OF THE INVENTION

[0001]

In the present invention, attention is focused on the matter that a thermophilic bacterium produced using a thermophilic bacterium exhibiting different functions depending on the diatheses of animal bodies and a mixed solution containing the thermophilic bacterium have a function to cause different physiological reactions depending on genetic backgrounds of the animal bodies and diatheses of an enterobacterial flora. Thus, the present invention relates to a preparation utilizing the function and a method for producing the preparation.

BACKGROUND OF THE INVENTION

[0002]

As preparations for controlling the intestinal function in an animal body in good condition, probiotics and prebiotics are known. As the techniques for the preparations, techniques which utilize microorganisms inhabitable predominantly at ambient temperature, such as lactic acid bacteria, yeast fungi and grass bacillus, have been widely used (Patent Literature 1, Patent Literature 2). For example, Patent Literature 1 discloses a method for producing a non-fermentative lactic acid bacterium using a bifidobacterium and Lactobacillus acidophilus; and Patent Literature 2 discloses a preparation for controlling the secretion of adiponectin using a culture supernatant of a lactic acid bacterium Lactobacillus gasseri SBT2055 (FERM BP-10953). Patent Literature 3 discloses an Anoectochilus spp. polysaccharide extract and a

pharmaceutical composition both for stimulating the growth of a bacterium belonging to the genus Bifidobacterium, stimulating the release of a granulocyte colony-stimulating factor, promoting the differentiation of T helper cell type I and/or suppressing the differentiation of T helper cell type II, and methods respectively for preparing the extract and the pharmaceutical composition, wherein the techniques relating to the Anoectochilus spp. polysaccharide extract and the pharmaceutical composition both for stimulating the growth of a bacterium belonging to the genus Bifidobacterium, stimulating the release of a granulocyte colony-stimulating factor, promoting the differentiation of T helper cell type I and/or suppressing the differentiation of T helper cell type II are introduced.

[0003]

As Non Patent Literatures, a worldwide study in which, among bacteria species belonging to the genus Clostridium bacteria species and bacteria both involved in the control of immune system cells are identified is known (Non Patent Literature 1). Also known are segmented filamentous bacteria (SFB) which are specific bacteria capable of regulating immune systems (Non Patent Literature 2) and an extremely important study for searching for an effective gene capable of protecting against O157 in a bifidobacterium (Non Patent Literature 3).

[0004]

In these techniques, however, the control of enterobacterial florae depending on the types of the enterobacterial florae with genetic backgrounds of hosts taken into consideration is not referred. In recent years, study data which demonstrate worldwide that both of feeds and sexual difference affect the constitution of an intestinal microbial flora have been reported (Non Patent Literature 4), and it has been shown that the diversity of an enterobacterial flora is important for the measure against metabolome in

human bodies (Non Patent Literature 5). Therefore, it is considered that it will be needed in the future to design a combination of proper probiotics on the basis of the genetic backgrounds of hosts.

[0005]

On the other hand, the present inventors have succeeded in the establishment of techniques for probiotics having influence on animal living bodies by using thermophilic bacteria which are one type of extremophiles capable of hardly proliferating in an ambient temperature range (Patent Literatures 4 and 5, and Non Patent Literature 5).

CITATION LIST

PATENT LITERATURES

[0006]

Patent Literature 1: Japanese Patent No. 4898859

Patent Literature 2: Japanese Patent No.5225652

Patent Literature 3: Japanese Patent No.5395733

Patent Literature 4: Japanese Patent No.5578375

Patent Literature 5: Japanese Patent No.5041228

NON PATENT LITERATURES

[0007]

Non Patent Literature 1: Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K*. "Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota." Nature 500:232-236. (2013)

Non Patent Literature 2: Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T,

Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K*, Littman DR*. "Induction of intestinal Th17 cells by segmented filamentous bacteria." Cell. 139:485-98. (2009)

Non Patent Literature 3: Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, Tobe T, Clarke JM, Topping DL, Suzuki T, Taylor TD, Itoh K, Kikuchi J, Morita H, Hattori M, Ohno H. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature. 2011 Jan 27;469(7331):543-7.

Non Patent Literature 4: Bolnick DI, Snowberg LK, Hirsch PE, Lauber CL, Org E, Parks B, Lusis AJ, Knight R, Caporaso JG, Svanbäck R Individual diet has sex-dependent effects on vertebrate gut microbiota. Nat Commun. 2014 Jul 29;5:4500 doi: 10.1038/ncomms5500.

Non Patent Literature 5: Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P, Li J, Burgdorf K, Grarup N, Jorgensen T, Brandslund I, Nielsen HB, Juncker AS, Bertalan M, Levenez F, Pons N, Rasmussen S, Sunagawa S, Tap J, Tims S, Zoetendal EG, Brunak S, Clement K, Dore J, Kleerebezem M, Kristiansen K, Renault P, Sicheritz-Ponten T, de Vos WM, Zucker JD, Raes J, Hansen T; MetaHIT consortium, Bork P, Wang J, Ehrlich SD, Pedersen O. Richness of human gut microbiome correlates with metabolic markers. Nature 500:541-549. (2013)

Non Patent Literature 6: Miyamoto H, Seta M, Horiuchi S, Iwasawa Y, Naito T, Nishida A, Miyamoto H, Matsushita T, Itoh K, Kodama H (2013) Potential probiotic the rmophiles isolated from mice after compost ingestion. Journal of Applied Microbiology,114(4): 1147-1157

SUMMARY OF THE INVENTION

[0008]

In the conventional techniques, the idea of administering different probiotics to different animals depending on diatheses of the animals has not been established yet. However, it is known that bacterial florae that form intestinal environments as well as genetic backgrounds vary greatly depending on the species of animals. Therefore, probiotics or prebiotics which can be utilized with the above-mentioned situations taken into consideration may be demanded.

[0009]

The diatheses of animals vary depending on genetic backgrounds of the animals, and the microbial structure of an enterobacterial flora inherent in a host and the behavior of the concentration of a biological molecule sometimes vary depending on the diathesis of the host. An antibiotic is sometimes used as a control for an enterobacterial flora. In this case, however, it is a concern that the diversity of an enterobacterial florae may be lost and an adverse effect on intestinal environments may develop. In these cases it is necessary to administer a proper probiotic.

[0010]

A thermophilic bacterium probiotic which can act depending on the properties of animals of strains having susceptibility to fatness and animals of strains having insusceptibility to fatness is used. By using this probiotic, the population of enterobacterial florae in a host is modified and the behavior of a physiological molecule in the liver is controlled properly. A thermophilic bacterium probiotic which can also act in the case where the properties of animal species or an aging phenomenon in the

intestine is observed is used. By using this thermophilic bacterium probiotic, the population of enterobacterial florae in a host is modified and the behavior of enterobacterial florae is controlled properly. For these reasons, a bacterium having Accession No. NITE BP-863, which is one of thermophilic Bacillus bacteria, is used. Accession No. NITE BP-863 has been internationally deposited by the present inventors with National Institute of Technology and Evaluation (NITE) Patent Microorganisms Depositary (NPMD) (#122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba, Japan) on January 15, 2010 (Accession No. BP-863).

[0011]

The present invention also includes a method for producing a preparation which can exert a function of a probiotic or prebiotic depending on diatheses even when the preparation is prepared using a mixture of microorganisms.

[0012]

According to an aspect of the invention there is provided a microbial preparation for controlling the proportion of the population of bacteria belonging to the division Bacteroidetes, Firmicutes or Proteobacteria or the proportion of the population of bacteria belonging to the genus Clostridium, Lactobacillus, Bifidobacterium or Bacteroidetes in enterobacterial florae in an animal body, and for controlling the concentration of a functional molecule contained in a living body, the microbial preparation containing a microorganism P01931 (Accession No. BP-1931; internationally deposited with National Institute of Technology and Evaluation (NITE) Patent Microorganisms Depositary (NPMD) (#122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba, Japan) on September 4, 2014), or MK-01A (Accession No. BP-02066; internationally deposited with National Institute of Technology and Evaluation (NITE) Patent Microorganisms Depositary (NPMD) (#122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba, Japan) on June

17, 2015), or MK-03A (Accession No. BP-02067; internationally deposited with National Institute of Technology and Evaluation (NITE) Patent Microorganisms Depositary (NPMD) (#122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba, Japan) on June 17, 2015), or a component of the microorganism.

[0013]

According to another aspect of the invention, there is provided a microbial preparation for reducing bacteria belonging to at least one genus selected from the genera Enterococcus, Streptococcus and Clostridium cluster XI that increases with age and fatness among opportunistic infection bacteria in enterobacterial florae confirmed in individual animal species, the microbial preparation containing a microorganism having Accession No. NITE BP-863 or a component of the microorganism having Accession No. NITE BP-863.

[0014]

In one embodiment there is provided a microbial preparation having a function recited in the present invention and capable of increasing Lactobacillus amylovorans in chickens, pigs and other animals.

[0015]

In another embodiment there is provided a microbial preparation having a function recited in the present invention and capable of increasing the diversity of a bacterial flora.

[0016]

In a further embodiment there is provided a microbial preparation having a function recited in the present invention and capable of controlling the function of enterobacterial florae and a physiological function in an animal body depending on a diathesis associated with susceptibility to fatness or insusceptibility to fatness.

[0017]

In a still further embodiment there is provided a health food capable of reducing the amount of an antibiotic to be used and exhibiting a diathesis-improving function depending on the diathesis of an animal body and a human body by utilizing a function recited in the present invention.

[0018]

In yet another embodiment there is provided a medicine capable of reducing the amount of an antibiotic to be used and exhibiting a diathesis-improving function depending on the diathesis of an animal body and a human body by utilizing a function recited in the present invention.

[0019]

In the context of the present application, it becomes possible to use a tailor-made type probiotic or prebiotic on the basis of genetic backgrounds of host animals, and therefore the spread of the probiotic or prebiotic is considered to be greatly effective. That is, for human bodies, it is conceived to deal depending on the difference in human races, the difference in districts, the difference in eating habits or the difference in disease types. In animals, it is obvious that the enterobacterial florae and diatheses vary depending on whether the animals are pet animals, farm animals or domestic poultry. Therefore, it becomes possible to establish a probiotic or prebiotic depending on the desired purpose with the above-mentioned differences taken into consideration. In addition, according to the present invention, it also becomes possible to control the microbial structure of an enterobacterial flora at ambient temperature inherent to hosts and the concentration of a biological molecule involved in a physiological function, depending on genetic backgrounds of animals, i.e., the property of a fatness-susceptible or fatness-insusceptible diathesis. Furthermore, it becomes

possible to develop and spread a probiotic which can act efficiently depending on the diatheses of animals. Furthermore, it also becomes possible to control the increase or decrease in diversity of an enterobacterial flora, particularly to reduce opportunistic infection bacteria, depending on the genetic background-related diatheses of animals or the microbial structure of the enterobacterial flora at ambient temperature inherent to the hosts, thereby maintaining the population of useful bacteria.

[0020]

We can increase the diversity of an enterobacterial flora significantly and particularly can reduce opportunistic infection bacteria in chickens, pigs and dogs by using a feed containing the BP-863. For example, the conventional problems can be solved by reducing the population of bacteria belonging to the genus Enteroccocus in chickens, the population of bacteria belonging to the genus Streptococcus in pigs and the population of bacteria belonging to the genus Clostoridium, which normally increases in old dogs, in dogs and by administering a species-specific useful bacterium, which tends to be decreased in these animal species, in combination. In this regard, the diatheses of animals vary depending on genetic backgrounds of the animals, and the microbial structure of an enterobacterial flora inherent in a host and the behavior of the concentration of a biological molecule sometimes vary depending on the diathesis of the host. In this case, it is needed to administer a proper probiotic. Thus, studies on diathesis-dependent probiotics which can control the diathesis-dependent properties are future challenges. There is also a case where an antibiotic is used as a control for an enterobacterial flora. In this case, the diversity of the enterobacterial flora may be lost and a possibility of the occurrence of adverse effects in intestinal environments is concerned. In this case, the administration of a proper probiotic is needed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021]

Fig. 1 shows a conceptual diagram of a diathesis-dependent probiotic.

Fig. 2 shows thermophilic bacteria which can be used in a diathesis-dependent probiotic and a standard bacterium strain for the thermophilic bacteria.

Fig. 3 shows the regulation of the glycolytic system in a liver using a diathesis-dependent probiotic.

Fig. 4 shows the regulation of β-oxidation and the TCA cycle in a liver using a diathesis-dependent probiotic.

Fig. 5 shows the regulation of the urea cycle in a liver using a diathesis-dependent probiotic.

Fig. 6 shows a conceptual diagram illustrating the control of enterobacterial flora without relying on the use of an antibiotic.

Fig. 7 shows a weight gain rate in a piglet receiving oral feeding of BP-863.

Fig. 8 shows a diversity of a bacterial flora in feces from a piglet receiving oral feeding of BP-863.

Fig. 9 shows the behavior of an opportunistic infection bacterium in feces from a piglet.

Fig. 10 shows the behavior of an opportunistic infection bacterium and a diversity of a bacterial flora in feces from a chicken.

DESCRIPTION OF EMBODIMENTS OF THE INVENTION

[0022]

Next, an embodiment of the present invention will be described. However, the present invention is not limited to the embodiments as described herein.

[0023]

Examples of the microorganism to be used in the present invention include

thermophilic microorganisms of multiple organism species. Specific examples of the organism species include Bacillus coagulans and Bacillus thermoamylovorans, and related species thereof. The microorganism to be used in the present invention is particularly preferably the microorganism having Accession No. NITE P-01931 and/or a microorganism having Accession No. NITE BP-1051 (internationally deposited with National Institute of Technology and Evaluation (NITE) Patent Microorganisms Depositary (NPMD) (2-5-8 Kazusakamatari, Kisarazu-shi, Chiba, Japan) on January 18, 2011) and/or the microorganism having Accession No. NITE BP-863 and/or a mixture of microorganisms MK-01 of which the accession was refused by National Institute of Technology and Evaluation (NITE) Patent Microorganisms Depositary (NPMD) because the strain was a mixture of microorganisms (Accession Refusal Notice No. 2014-0319) and/or a mixture of microorganisms MK-03 of which the accession was refused by National Institute of Technology and Evaluation (NITE) Patent Microorganisms Depositary (NPMD) because the strain was a mixture of microorganisms (Accession Refusal Notice No. 2014-0321). The species relating to Bacillus thermoamylovorans, particularly the group of microorganisms to be used in the present invention is preferably microorganisms having Accession No. NITE BP-863.

[0024]

Examples of the microorganism that can be mixed with the microbial material to be used in the present invention include microorganisms belonging to the genera Lactobacillus and Bifidobacterium and thermophilic microorganisms belonging to the genera Bacillus, Lysinibacillus, Virgibacillus, Anoxybacillus and Paenibacillus. The desired physiological activity can also be achieved when Thermophiles inoculum MIROKU H2K including microorganisms belonging the genera Meiothermus, Vulcanithermus, Thermus and Oceanobacillus in the division Deinococcus-Thermus are

co-present. The accession of this group of microorganisms Thermophiles inoculum MIROKU H2K was refused in National Institute of Technology and Evaluation (NITE) Patent Microorganisms Depositary (NPMD) because the strain was a mixture of microorganisms and could not be cultured easily, and therefore have been stored in Miroku Co., Ltd. (Kitsuki-shi, Ohita, Japan). As the group of microorganisms that can be co-present, microorganisms having Accession No. PTA-1773 which have been internationally deposited with ATCC (American Type Culture Collection, 10801 University Boulevard Manassas, Virginia 20110-2209, USA) on May 1, 2000 can also be used. In addition, microorganisms that have been deposited with National Institute of Technology and Evaluation (NITE) Patent Microorganisms Depositary (NPMD) as a mixture of microorganisms under Accession No. NITE BP-1051 can also be used.

[0025]

The Accession No. PTA-1773 is a thermophilic bacteria species.

[0026]

In the preparation according to the present invention, a microorganism of each of the above-mentioned bacteria strains or a functional component derived from the microorganism is preferably contained in an amount of about 10 cells/g to about 10^9 cells/g.

[0027]

We have found that a thermophilic bacterium causes different physiological reactions in mice of different strains, using the above-mentioned microorganisms. When cells of each of the bacterium are administered to a mouse of a fatness-susceptible strain to a mouse of a fatness-insusceptible strain, the populations of bacteria belonging to the division Bacteroidetes, bacteria belonging to the genus Clostridium and bacteria belonging to the genus Lactobacillus in a microflora in the

intestine can be controlled and the concentration of a functional molecule in a liver can also be controlled. In bacteria belonging to a single strain, there are a bacterial strain that tends to exert the effect thereof both on mice of a fatness-susceptible strain and mice of a fatness-insusceptible strain in the same manner and a bacterial strain that tends to exert the effect thereof on mice of a fatness-susceptible strain and mice of a fatness-insusceptible strain in a quite opposite manner. By utilizing these natures, the bacteria are used as diathesis-dependent probiotics and consequently the conventional problems can be solved.

[0028]

The diversity of an enterobacterial flora can be increased significantly and particularly opportunistic infection bacteria can be reduced utilizing the above-mentioned microorganisms and by using a feed containing the BP-863 in chickens, pigs and dogs. For example, the conventional problems can be solved by reducing the population of bacteria belonging to the genus Enteroccocus in chickens, the population of bacteria belonging to the genus Streptococcus in pigs and the population of bacteria belonging to the genus Clostoridium, which normally increases in old dogs, in dogs. By utilizing these natures, the bacteria are used as probiotics capable of controlling an enterobacterial flora without relying on an antibiotic.

[0029]

(Example 1)

In a breeding test under a high-fat diet, the test was carried out using the formulations shown in Table 1.

[0030]

[Table 1]

Table1

Formulation		High-fat diet(lard)		High-fat diet	
General components	Moisture	6.2	g	6.2	g
	Protein	18.9	g	25.5	g
	Fat	24.2	g	32	g
	Ash	4.9	g	4	g
	Fiber	2.3	g	2.9	g
	Carbohydrate	43.5	g	29.4	g
	Total	100	g	100	g

※ High-fat diet (lard) contained 20% of lard

[0031]

BALB/c and C57BL/6 mice (male, three-week old) were introduced and then raised preliminarily for 5 days, and then an experiment started. The four groups (1) to (4) mentioned below were provided for each of the mouse strains, i.e., eight groups in total: (1) a group raised with a high-fat diet (lard) (a control group) (symbol mA for BALB/c) (symbol mE for C57BL/6); (2) a group raised with a high-fat diet (lard) + with the addition of a thermophilic bacterium MK01A solution to drinking water (symbol mB for BALB/c) (symbol mF for C57BL/6); (3) a group raised with a high-fat diet (lard) + with the addition of a thermophilic bacterium P01931 solution to drinking water (symbol mC for BALB/c) (symbol mG for C57BL/6); and (4) a group raised with a high-fat diet (lard) + with the addition of a thermophilic bacterium MK03A solution to drinking water (symbol mD for BALB/c) (symbol mH for C57BL/6). A group of mice, which was composed of five mice, was raised in one cage. A formulated feed (MF manufactured by Oriental Yeast Co., Ltd.™) was used as a standard feed, and the high-fat diet was produced by KBT Oriental Co., Ltd. (Tosu-shi, Saga, Japan), in which the fat content was adjusted to 24% (wherein lard made up 20%). The mice were allowed to take tap water ad libitum as the drinking water. In groups other than the control group,

a 1.0% solution of the corresponding thermophilic bacterium was added to the drinking water. The mice were allowed to take a feed ad libitum within an intake limitation of 25 g per day. The mice were raised for 2 months, and were then subjected to the measurement of body weights, the collection of blood or the like, anatomy and the collection of livers and feces. The livers and feces were subjected to a metabolomic analysis and a bacterial flora analysis. The bacteria strains are shown in Fig. 2. Genetically, a 16SrDNA sequence in each of the bacteria strains was exactly the same as that in Bacillus coagulans (ATCC™) that is a standard strain. However, the bacteria strains were different from each other morphologically.

[0032]

As the results of the bacterial flora analysis, in the BALB/c mice, the change in bacterial flora in feces was confirmed in four groups, as shown in Tables 2 and 3. Especially in the mD group in which the body weight increasing tendency was low, a tendency that the populations of the bacteria belonging to the genera Clostridium and Lactobacillus were increased was confirmed.

[0033]

[Table 2]

Table2 Bacterial flora data of division level

BALB/c	Average			
Phylum	mA Cf	mB Cf	mC Cf	mD Cf
Firmicutes	1201.75	928.25	1433.5	1916.25
Actinobacteria	935.25	967.5	367	252.75
Bacteroidetes	367.5	723	652	438.5
Proteobacteria	14.5	25.25	40.75	40
Deferribacteres	1.25	5.5	12.5	4.25

[0034]

[Table 3]

Table 3 Bacterial flora data of genus level

BALB/c	Average			
Genus	mA Cf	mB Cf	mC Cf	mD Cf
Clostridium	950.5	518	738.25	1248.25
Bifidobacterium	887.5	868.25	313	196.25
Lactobacillus	36.5	60.25	81.75	199.25
Bacteroides	28	86	83	40.25
Robinsoniella	8.25	18	16	18.25

[0035]

As the results of the bacterial flora analysis, in the C57BL/6 mice, the change in bacterial flora in feces was confirmed in four groups, as shown in Tables 4 and 5. Especially in the mG group in which the body weight increasing tendency was low, a tendency that the populations of the bacteria belonging to the genera Clostridium and Lactobacillus were increased was confirmed.

[0036]

[Table 4]

Table 4

C57BL/6	Average			
Phylum	mE Cf	mF Cf	mG Cf	mH Cf
Firmicutes	1351.5	1273	1676	1365
Bacteroidetes	897.25	937	739.5	715.25
Actinobacteria	41.75	107.25	27	43
Proteobacteria	23.5	24	8.5	14.75
Verrucomicrobia	7.25	0.75	4	1.25

[0037]

[Table 5]

Table 5

C57BL/6	Average			
Genus	mE Cf	mF Cf	mG Cf	mH Cf
Lactobacillus	593	137.75	793	160.75
Clostridium	19.25	66.75	43.75	54.5
Robinsoniella	44.25	73.25	41.5	17
Bacteroides	45	47.25	27.25	29
Bifidobacterium	7.25	81	2.75	18.5

[0038]

The analysis on diversity of the bacterial flora was confirmed by two kinds of methods. As a result, it was found that the diversity tended to increase in both of the bacteria strain administration groups, as shown in Tables 6 and 7, and therefore both of the groups were not different from each other with respect to this matter.

[0039]

[Table 6]

Table 6 Diversity analysis of bacterial flora

Index	mA (control)	mB (MK-01A)	mC (P01931)	mD (MK-03A)
OTU number	100	111	132	124
Chao1	100	103	116	132

[0040]

[Table 7]

Table 7 Diversity analysis of bacterial flora

Index	mE (control)	mF (MK-01A)	mG (P01931)	mH (MK-03A)
OTU number	100	121	106	116
Chao1	100	116	100	114

[0041]

Next, the metabolomic analysis on a liver was carried out by CE-MS. As a result, the concentrations of biological functional molecules were different among the groups, as shown in Table 8. The results were not necessarily the same in some of the strains. In the analysis with respect to the glycolytic system, the decreasing tendency was confirmed in the production of F6P and G6P regardless of the types of the strains of the mice, as shown in Fig. 3. In contrast, in the analysis with respect to the TCA cycle, the increasing tendency was confirmed only in the mD group to which the MK03A strain was administered, but no change was observed in the other groups.

[0042]

[Table 8]

Table 8 Metabolomic analysis data on a liver

	BALB/c 3W				C57BL/6 3W			
Functional molecule in a liver	Control	MK01A	P01931	MK03A	Control	MK01A	P01931	MK03A
Allantoin	100	98	76	90	100	101	104	100
alpha-Aminoadipate	100	71	78	74	100	98	82	99
Betaine	100	73	98	173	100	105	95	71
Carnitine	100	92	93	119	100	102	99	97
cis-Aconitate	100	98	87	138	100	95	102	87
Choline	100	107	120	151	100	90	103	120
F6P	100	103	73	27	100	85	86	42
G6P	100	110	70	30	100	106	91	39
GABA	100	92	111	176	100	174	306	385
Gluconate	100	125	120	142	100	92	89	106
Hypoxanthine	100	123	137	196	100	125	166	177
Inosine	100	112	116	115	100	126	125	105
Isethionate	100	113	119	168	100	98	108	105
Lys	100	101	113	141	100	119	151	186
Met	100	95	102	204	100	118	151	175
N-Acetylglucosamine	100	110	135	231	100	147	232	262
N-Acetylglutamate	100	77	61	81	100	90	69	73
Pantothenate	100	121	129	203	100	142	187	254
Thiamine	100	136	145	176	100	111	119	107
Xanthine	100	109	122	163	100	117	135	161

[0043]

BALB/c and C57BL/6 mice (male, three-week old; and male, eight-week old) were introduced and then raised preliminarily for 5 days, and then an experiment started. The three groups (1) to (3) mentioned below were provided: (1) a group raised in a normal manner (a control group); (2) a group with the addition of the BP-863; and (3) a group with the addition of a mixed solution to drinking water. A group of mice, which was composed of five mice, was raised in one cage. The mice were allowed to take tap water ad libitum as the drinking water, and were also allowed to take a feed ad libitum. The mice were raised for 3 months, and were then subjected to the measurement of body weights, the collection of blood or the like, anatomy and the collection of livers. The liver was subjected to a metabolomic analysis.

[0044]

As the result of the blood analysis, the concentrations of leptin in serum in the C57BL/6 mice were higher than those in the BALB/c mice, but the C57BL/6 mice were likely to gain much body weights compared with the BALB/c mice.

[0045]

As the result of the metabolomic analysis on livers, the same tendency was not observed depending on the age in weeks of the mice at the time of starting of administration and the strain of the mice, as shown in Tables 9 and 10.

[0046]

[Table 9]

Table 9

Functional molecule in a liver	B6 3w			BALB/c 3w		
	High-fat diet	BP-863	Mixed slution	High-fat diet	BP-863	Mixed slution
Allantoin	100	96	109	100	123	101
alpha-Aminoadipate	100	289	144	100	178	147
Betaine	100	91	142	100	146	161
Carnitine	100	130	118	100	110	103
Choline	100	86	95	100	118	116
cis-Aconitate	100	217	377	100	469	498
F6P	100	101	61	100	88	75
G6P	100	94	57	100	100	76
GABA	100	76	104	100	67	64
Gluconate	100	88	92	100	112	132
Hypoxanthine	100	81	94	100	82	151
Inosine	100	91	93	100	108	95
Isethionate	100	106	82	100	133	114
Lys	100	91	98	100	105	138
Met	100	86	121	100	89	112
N-Acetylglucosamine	100	84	89	100	78	87
N-Acetylglutamate	100	171	139	100	190	170
Pantothenate	100	72	71	100	110	88
Thiamine	100	82	92	100	119	111
Xanthosine	100	93	102	100	109	104

[0047]

[Table 10]

Table 10

Functional molecule in a liver	B6 8w			BALB/c 8w		
	High-fat diet	BP-863	Mixed slution	High-fat diet	BP-863	Mixed slution
Allantoin	100	94	98	100	98	87
alpha-Aminoadipate	100	104	139	100	153	152
Betaine	100	70	53	100	144	128
Carnitine	100	99	112	100	97	97
Choline	100	106	120	100	161	120
cis-Aconitate	100	98	120	100	447	1076
F6P	100	94	79	100	62	51
G6P	100	98	86	100	71	59
GABA	100	133	158	100	78	90
Gluconate	100	48	108	100	145	111
Hypoxanthine	100	111	139	100	97	102
Inosine	100	101	116	100	99	109
Isethionate	100	230	252	100	80	89
Lys	100	91	126	100	134	150
Met	100	95	118	100	119	111
N-Acetylglucosamine	100	91	136	100	98	121
N-Acetylglutamate	100	97	158	100	152	122
Pantothenate	100	29	19	100	120	104
Thiamine	100	116	143	100	99	87
Xanthosine	100	111	133	100	116	137

[0048]

The above results indicate that almost similar physiological reactions were induced by the bacterial species under a high-fat diet condition both in the mice each having a fatness-susceptible diathesis (C57BL/6) and the mice each having a fatness-insusceptible diathesis (BALB/c). As shown in Table 1, it was found that almost similar physiological reactions, including the increase in bacteria belonging to the division Bacteroidetes in the microflora in the intestine and the suppression of the glycolytic system, the activation of the TCA cycle and the activation of the urea cycle in

the liver, were induced. The increase in free amino acids was confirmed only in the mice of a fatness-insusceptible strain. The reaction was observed significantly in newborn three-week-old mice, while a slightly different reaction was observed in the eight-week old mice.

Therefore, it was concluded that the technique of the present invention is novel over the prior art.

[0049]

(Example 2)

A feed was prepared by blending the BP-863 to a feed in an amount of 10^2 per 1 kg of the feed, and the resultant feed was fed for 3 weeks to piglets which were born from the same mother pig and were about 30 day old from the time of birth.

[0050]

As a result, it was confirmed that the weight gain rate in the piglets tended to increase by about 5% (see Fig. 7). At this point of time, total DNA was extracted from porcine feces, and then subjected to the comprehensive analysis on bacterial 16SrDNA using a next-generation sequencer. That is, the analysis of a bacterial flora in porcine feces was carried out with respect to bacterial 16SrDNA using a next-generation sequencer through a 3000 read analysis. As a result, a change in bacterial flora was confirmed between pigs to which a feed containing the BP-863 was fed and pigs in a non-administered group, and the population of opportunistic infection bacteria was remarkably reduced. On the other hand, the number of detected bacterial species was significantly increased in the BP-863-fed zone in the 3000 reads. This analysis was carried out employing the method described in DNA Res. Jun; 20(3): 241-253, 2013 and the method described in DNA Res. Feb; 21(1): 15-25, 2014.

[0051]

Specifically, as the result of the UniFrac analysis, the bacterial flora of the BP-863-unadministered group and the bacterial flora of the BP-863-administered group were significantly different from each other, as shown in Fig. 8. In addition, the number of detected units having different sequences (OUT) was increased in the BP-863-administered group, which demonstrates that diversity was increased. As the result of the detailed analysis, the population of bacterial species related to bacteria belonging to the genus Streptococcus which is assumed to be an opportunistic infection bacterium was remarkably reduced. It was also confirmed that the population of bacterial species belonging to cluster XI, among the genus Clostoridium, tended to be reduced. An example of the bacterium belonging to the genus Clostoridium, cluster XI is Clostridium mayombei. Clostoridium cluster XI is known as one of bacteria that have been assumed to be increased during the experience of fatness in an experiment using mice (Nature Jul 4; 499(7456): 97-101, 2013).

[0052]

As the bacteria that belog to the genus Streptococcus were also expected to be reduced in pigs in other experiment systems, Streptococcus alactoriticus, Streptococcus galactotiticus, Streptococcus orisuis and Streptococcus hyointestinalis were conceived.

[0053]

In the experiment, Lactobacillus amylovorus and a bacterium belonging to the genus Bifidobacterium, among lactic acid bacteria, tended to be increased.

[0054]

In general, the increase in the population of bacterial species belonging to the genus Clostridium in the intestine is observed with age. The tendency of reduction in Clostridium XI in the enterobacterial florae in an animal body receiving the

administration of BP-863 was also confirmed in old dogs.

[0055]

(Example 3)

Each of a feed containing the BP-863 and a feed not containing the BP-863 was fed to big chicks of egg-laying chickens for 18 weeks, and the bacterial flora in feces from each of the chicks was analyzed in the same manner as described in paragraph [0050].

[0056]

The analysis of a bacterial flora in chicken feces was carried out with respect to bacterial 16SrDNA using a next-generation sequencer through a 1800 read analysis. As a result, a change in bacterial flora was confirmed between chickens to which a feed containing the BP-863 was fed and chickens of a non-administered group, and bacteria belonging to the genus Enterococuus, which are known as vancomycin-resistant bacteria, were remarkably reduced. On the other hand, the number of detected bacterial species was significantly increased in the BP-863-fed zone among the 1800 reads. Specifically, a significant difference in bacterial flora was confirmed between the BP-863-administered group and the BP-863-unadministered group, as shown in Fig. 10. In addition, the number of units having different sequences (OUT) was increased in the BP-863-administered group, which demonstrates that diversity was increased. Particularly, the population of Enterococcus gallinarum, which is one of bacteria belonging to the genus Enterococcus, was remarkably reduced. In this experiment, the tendency of increase in the population of Lactobacillus amylovorus was confirmed.

[0057]

From these results, it was confirmed that the BP-863 has a tendency to control a bacterial flora and particularly increase the diversity of the bacterial flora, while it was

demonstrated that the BP-863 increases the population of specific useful bacteria and promotes the decrease in population of specific opportunistic infection bacteria. When an antibiotic is administered, this tendency causes the death of many enterobacterial florae and therefore causes the loss of diversity of a bacterial flora. Therefore, it can be considered that the technique of the present invention is novel over the prior art. As mentioned above, in recent years, it has been found that the diversity of an enterobacterial flora is essential for the control of obesity or the prevention of various diseases. Therefore, it is considered that the effectiveness of the present invention is high. According to the present invention, it is expected that it becomes possible to reduce specific opportunistic infection bacteria without relying on the use of antibiotics, and it also becomes possible to produce the diversity of an enterobacterial flora regardless of the types of species of animals to prevent various diseases.

[Accession Numbers]

[0058]

NITE BP-01931

[0059]

NITE BP-02066

[0060]

NITE BP-2067

[0061]

NITE BP-863

[0062]

NITE BP-1051

[0063]

ATCC PTA-1773

[0064]

Accession Refusal Notice No. 2014-0319

[0065]

Accession Refusal Notice No. 2014-0321

[0066]

Thermophiles inoculum MIROKU M2K strain

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A microbial preparation when used to increase the diversity of enterobacterial flora in an animal, wherein said microbial preparation comprises the microorganism MK-01A deposited under NITE BP-02066.

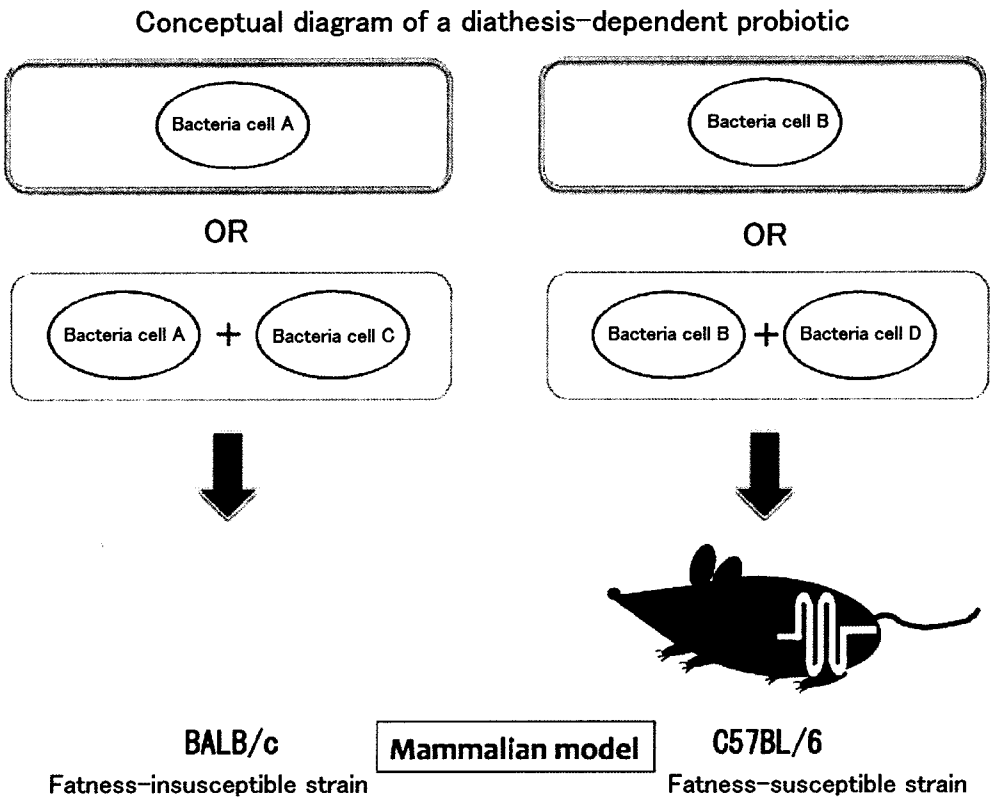
2. Use of the microbial preparation according to claim 1 to increase the diversity of enterobacterial flora in an animal.

3. A health food comprising the microbial preparation according to claim 1, when used to increase the diversity of enterobacterial flora in an animal.

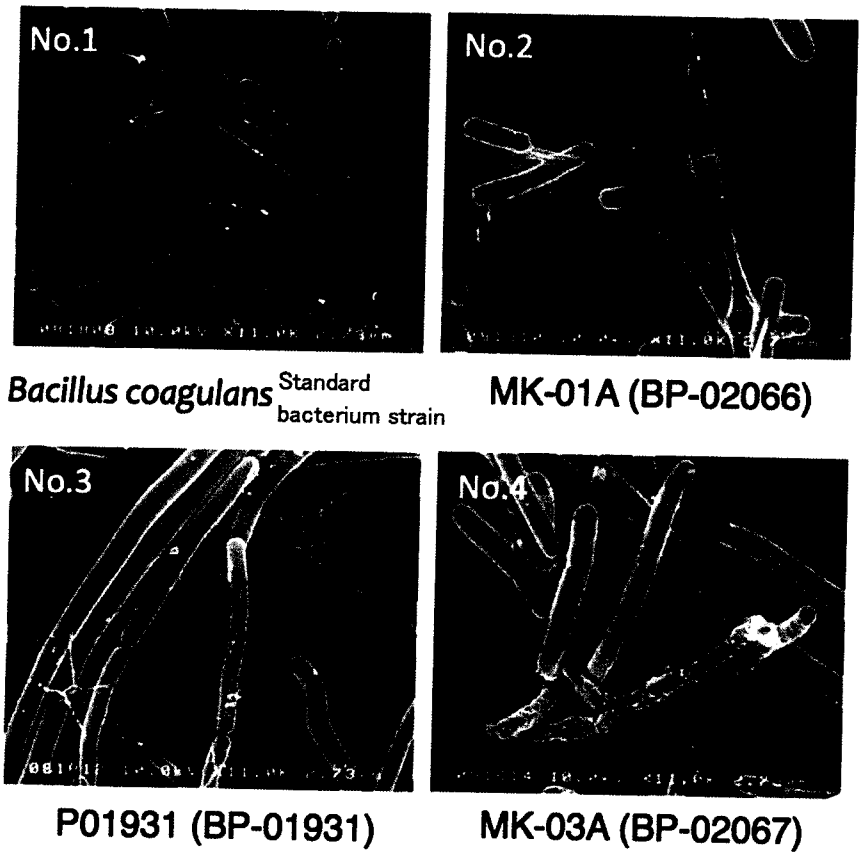
4. A medicine comprising the microbial preparation according to claim 1, when used to increase the diversity of enterobacterial flora in an animal.

Date Reçue/Date Received 2021-04-14

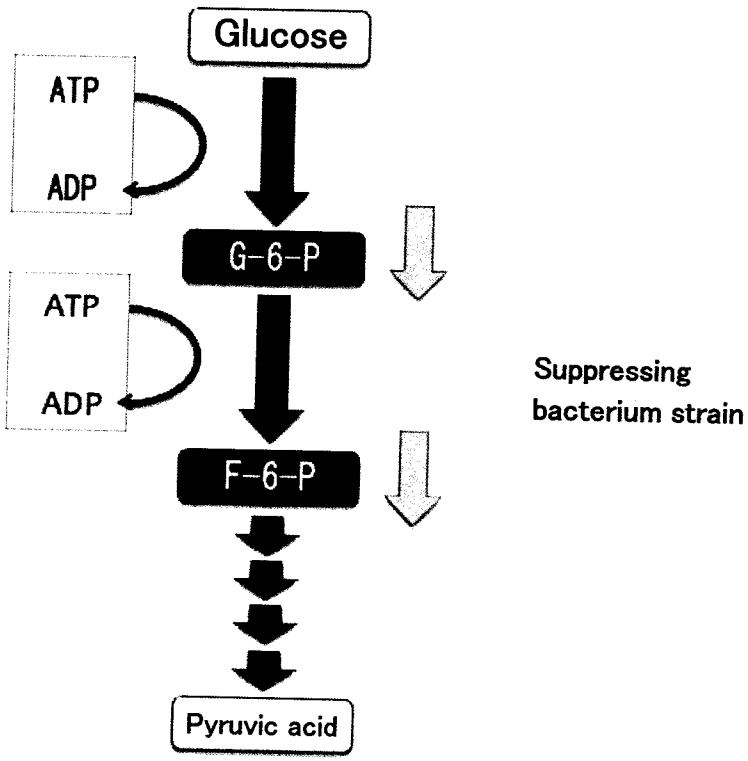
[Fig.1]


Conceptual diagram of a diathesis-dependent probiotic
Bacteria cell A
Bacteria cell B
OR
OR
Bacteria cell A
+
Bacteria cell C
Bacteria cell B
+
Bacteria cell D
BALB/c
Fatness-insusceptible strain
Mammalian model
C57BL/6
Fatness-susceptible strain

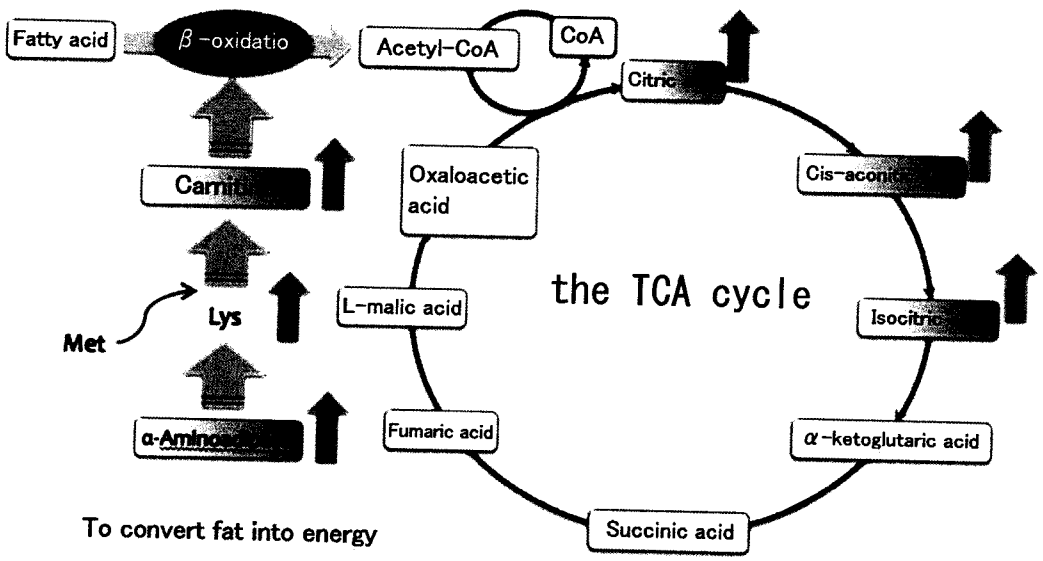
[Fig.2]


No.1
No.2
Bacillus coagulans Standard bacterium strain
MK-01A (BP-02066)
No.3
No.4
P01931 (BP-01931)
MK-03A (BP-02067)

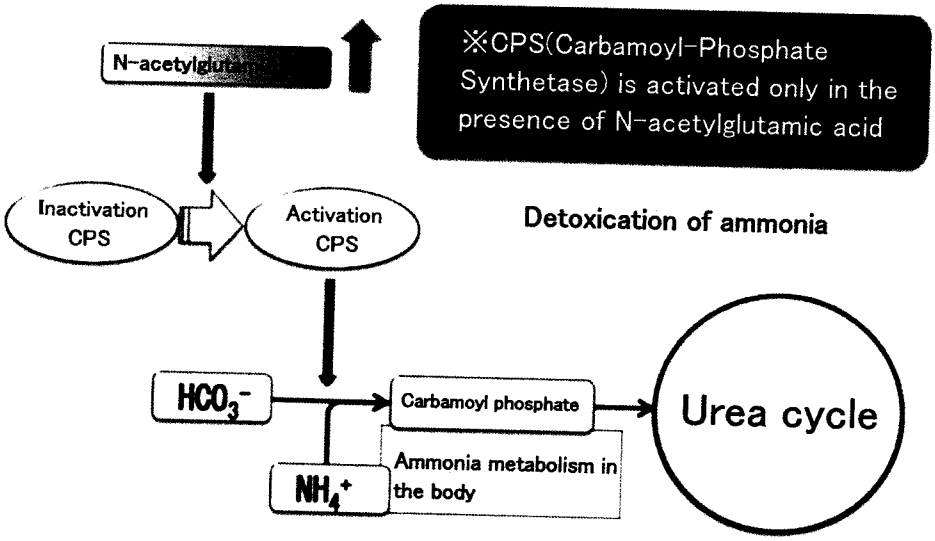
[Fig.3]


Glucose
ATP
ADP
G-6-P
ATP
ADP
F-6-P
Pyruvic acid
Suppressing
bacterium strain

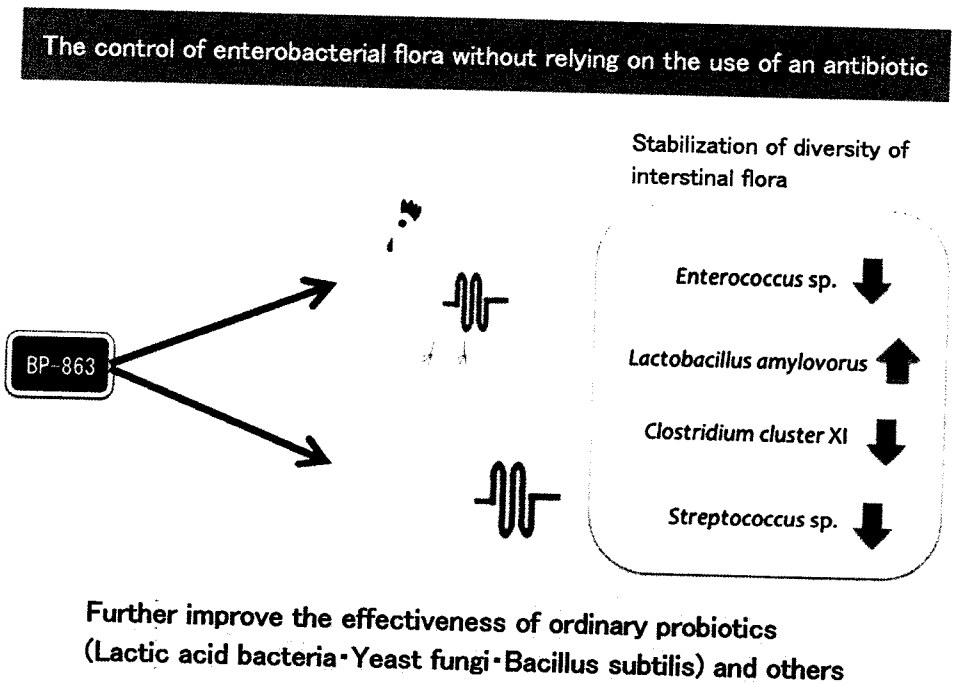
[Fig.4]


Fatty acid
β-oxidatio
Acetyl-CoA
CoA
Citric
Oxaloacetic
acid
Cis-aconic
Carni
Met
Lys
L-malic acid
the TCA cycle
Isocitric
α-Amino
Fumaric acid
α-ketoglutaric acid
Succinic acid
To convert fat into energy

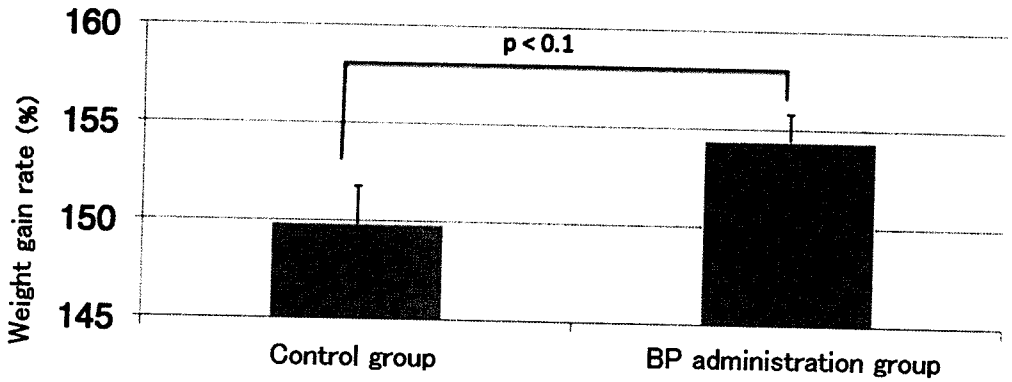
[Fig.5]


N-acetylglutamic
※CPS(Carbamoyl-Phosphate Synthetase) is activated only in the presence of N-acetylglutamic acid
Inactivation CPS
Activation CPS
Detoxication of ammonia
HCO_3^-
Carbamoyl phosphate
Urea cycle
NH_4^+
Ammonia metabolism in the body

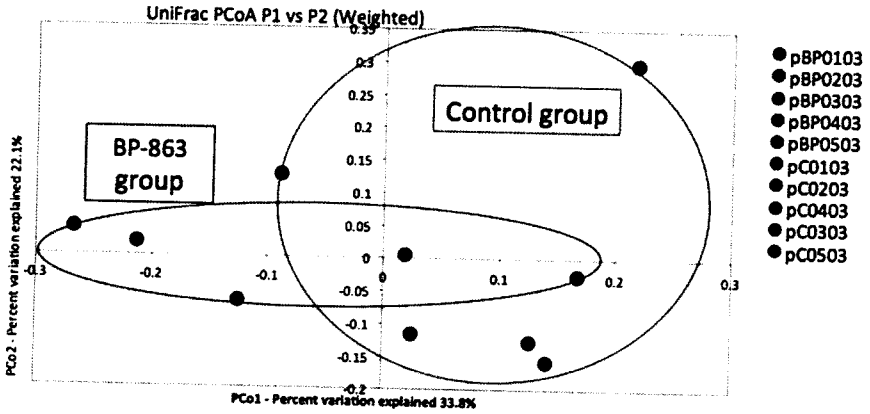
[Fig.6]


The control of enterobacterial flora without relying on the use of an antibiotic
Stabilization of diversity of interstinal flora
BP-863
Enterococcus sp.
Lactobacillus amylovorus
Clostridium cluster XI
Streptococcus sp.
Further improve the effectiveness of ordinary probiotics
(Lactic acid bacteria・Yeast fungi・Bacillus subtilis) and others

[Fig.7]



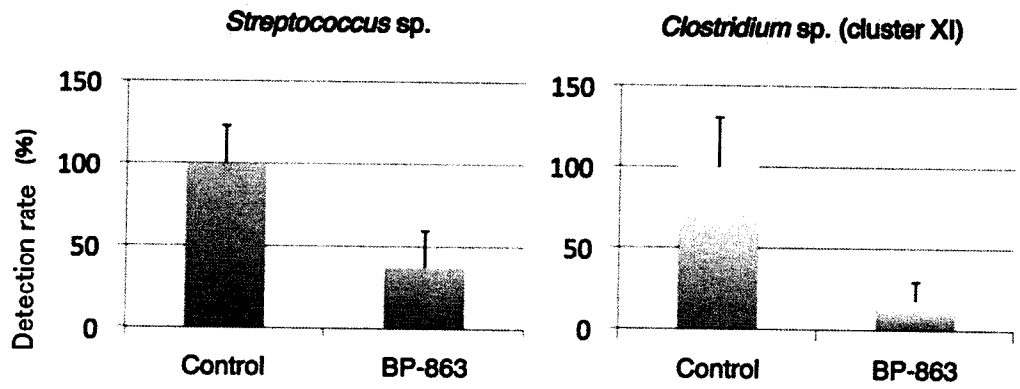
[Fig.8]



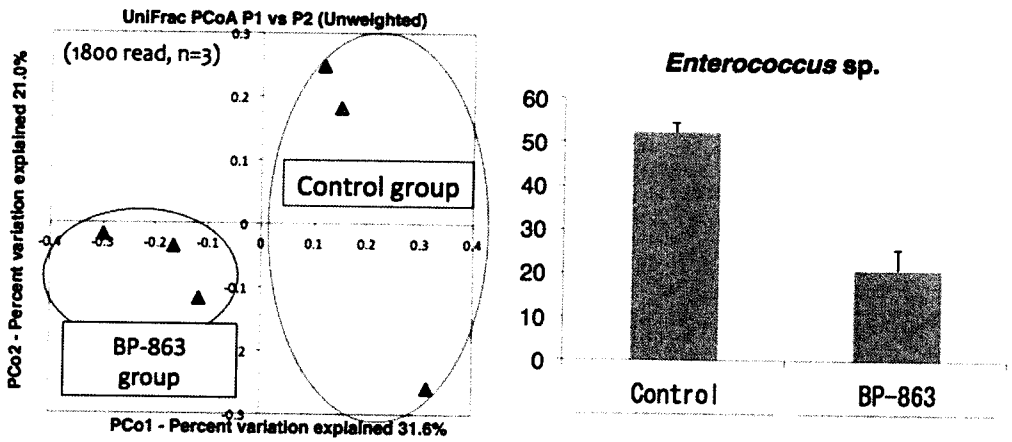
(mean±SE, %)

Richness	Control	BP-863	p value
Observed OTUs number	100.0±2.8	125.7±6.1	0.0097

[Fig.9]

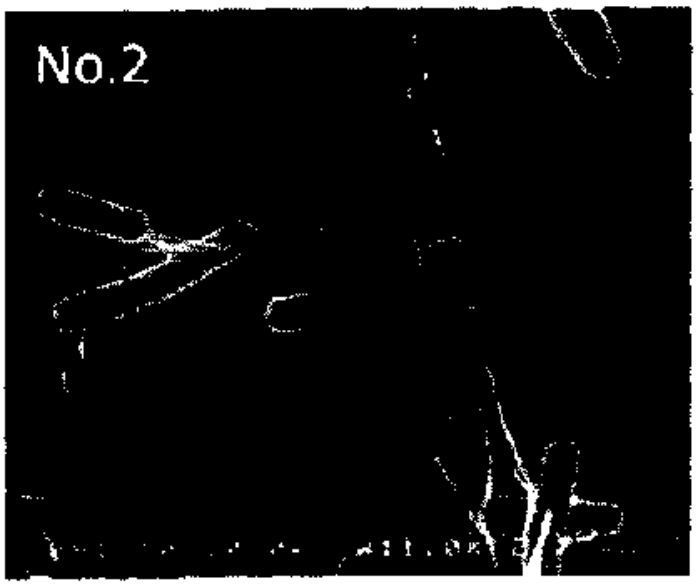


[Fig.10]



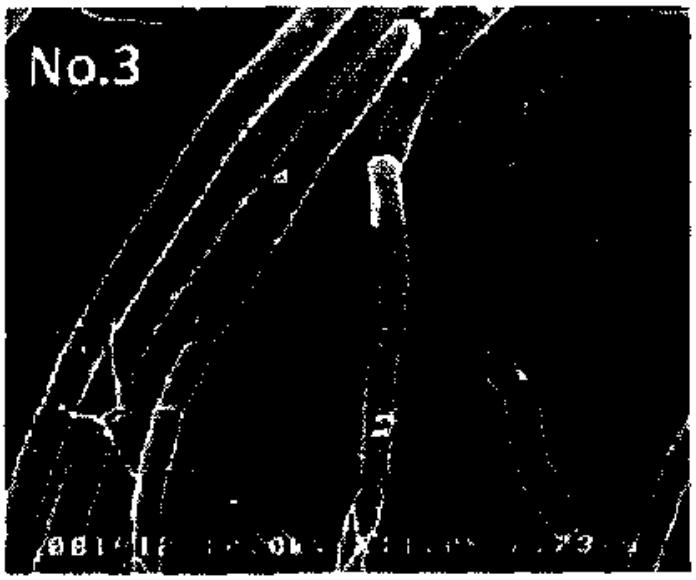
(mean±SE, %)

Richness	Control	BP-863 feed	p value
Observed OTUs number	100.0±2.9	134.1±7.1	0.0278



Bacillus coagulans Standard bacterium strain

MK-01A (BP-02066)



P01931 (BP-01931)

MK-03A (BP-02067)