

DESCRIPTION

Title of Invention

FOOD COMPOSITION THAT REDUCES AMOUNT OF MYCOTOXIN ABSORBED
INTO BODY

Technical Field

[0001]

The present invention relates to a food composition that reduces the amount of mycotoxin absorbed into a body.

Background Art

[0002]

Mycotoxin is a chemical substance produced by mold that contaminates food or feed. When food or feed contaminated with mycotoxin is taken into a body, serious health damages are caused to humans and livestock.

[0003]

For example, aflatoxin produced by certain mold belonging to the genus *Aspergillus* is a harmful substance with strong carcinogenicity. Since aflatoxin is colorless, odorless, and tasteless, humans and livestock cannot detect aflatoxin by sensory organs thereof, and there is a risk that humans and livestock are unknowingly exposed to a large amount of aflatoxin. In addition, since aflatoxin is physically and chemically stable and remains in food and feed even after mold is killed, it is difficult to remove

aflatoxin by an approach such as heating or washing.

[0004]

Under such a circumstance, in recent years, an attempt has been made to remove mycotoxin such as aflatoxin by biocontrol using useful microorganisms such as good bacteria. Non Patent Literature 1 discloses that ability to adsorb aflatoxin is improved by heat-inactivating a *Lactobacillus rhamnosus* strain in an in vitro test.

Citation List

Non Patent Literature

[0005]

Non Patent Literature 1: Effect of digestion and thermal processing on the stability of microbial cell-aflatoxin B₁ complex

Summary of Invention

Technical Problem

[0006]

However, there is no knowledge about a food and drink product that reduces the amount of mycotoxin once absorbed into a body, and there remains room for research and development.

[0007]

Therefore, an object of the present invention is to

provide a food composition that reduces the amount of mycotoxin absorbed into a body.

Solution to Problem

[0008]

As a result of intensive studies, the present inventors have found that a food composition containing lactic acid bacteria is effective in reducing the amount of mycotoxin absorbed into a body, and have completed the present invention. That is, the present invention is as follows.

[0009]

[1] A food composition comprising lactic acid bacteria as an active ingredient, wherein the food composition reduces an amount of mycotoxin absorbed into a body.

[2] The food composition according to [1], wherein the mycotoxin is aflatoxin.

[3] The food composition according to [2], wherein the food composition reduces the amount of aflatoxin in blood or in urine in the body.

[4] The food composition according to [3], wherein the lactic acid bacteria are administered to a subject at a dose of 5×10^9 to 2×10^{11} cells per day for at least six weeks.

[5] The food composition according to [3], wherein aflatoxin detected in urine is aflatoxin M₁, and aflatoxin detected in blood is aflatoxin B₁.

[6] The food composition according to [3], wherein the subject is a human or livestock.

[7] The food composition according to any one of [1] to [6], wherein the lactic acid bacteria are administered for at least ten weeks.

[8] The food composition according to any one of [1] to [6], wherein the lactic acid bacteria are administered for at least 12 weeks.

[9] The food composition according to any one of [1] to [6], wherein the food composition is a beverage further comprising a carbohydrate and a milk protein.

[10] The food composition according to any one of [1] to [6], wherein the lactic acid bacteria belong to the genus *Lacticaseibacillus*.

[11] The food composition according to [10], wherein the lactic acid bacteria are *Lacticaseibacillus paracasei* YIT 9029 (FERM BP-1366).

[12] A mycotoxin-reducing agent comprising *Lacticaseibacillus paracasei* as an active ingredient, wherein the agent reduces an amount of mycotoxin absorbed into a body.

[13] The mycotoxin-reducing agent according to [12],

wherein the *Lacticaseibacillus paracasei* is
Lacticaseibacillus paracasei YIT 9029 (FERM BP-1366).

Advantageous Effects of Invention

[0010]

According to the present invention, it is possible to provide a food composition that contains lactic acid bacteria as an active ingredient and reduces the amount of mycotoxin absorbed into a body.

Brief Description of Drawings

[0011]

Fig. 1 is a diagram for explaining a test schedule in Examples of the present invention.

Fig. 2 is a table for explaining the amount of aflatoxin in urine in Examples of the present invention.

Fig. 3 is a table for explaining the amount of aflatoxin in blood in Examples of the present invention.

Fig. 4 is a table presenting a relationship between the amount of aflatoxin in urine or in blood and a food ingested by a subject in Examples of the present invention.

Description of Embodiments

[0012]

Hereinafter, the present invention will be described

in detail. Description of constituent elements described below is an example as an embodiment of the present invention, and the present invention is not specified by these contents.

[0013]

The present invention has an effect of reducing the amount of mycotoxin absorbed into a body by allowing a subject to ingest/administer a food composition containing lactic acid bacteria as an active ingredient at a predetermined dose for a predetermined period.

[0014]

The food composition of the present invention has an application of a food composition for reducing the amount of mycotoxin, a food composition for removing mycotoxin, or a food composition for inhibiting mycotoxin absorption, which reduces the amount of mycotoxin absorbed into a body. The present invention is intended to be used for a human or livestock. Furthermore, the present invention has an application as a mycotoxin reducing agent, a mycotoxin removing agent, or a mycotoxin absorption inhibitor, reducing the amount of mycotoxin absorbed into a body.

[0015]

Here, the "mycotoxin absorbed into a body" refers to mycotoxin absorbed from cells of a digestive organ through oral ingestion and taken into a body, or a metabolite of

the mycotoxin.

The mycotoxin absorbed into a body can be detected from a blood sample or a urine sample sampled from a subject.

[0016]

Examples of "mycotoxin" include aflatoxin, ochratoxin A, deoxynivalenol, nivalenol, and patulin, and particularly include aflatoxin. Examples of aflatoxin detected in urine or in blood include aflatoxin M₁ (aflatoxin M₁ or AFM₁) and aflatoxin B₁ (aflatoxin B₁ or AFB₁). Aflatoxin M₁ is a metabolite obtained by decomposition of aflatoxin B₁ taken into a body in the liver.

[0017]

In general, AFM₁ is known as a marker reflecting aflatoxin ingestion in the last few days, and is used as a marker detected from urine. On the other hand, AFB₁ is known as a marker reflecting aflatoxin ingestion in the last two to three months, and is used as a marker detected from blood.

[0018]

As for the food composition containing lactic acid bacteria as an active ingredient of the present invention, it is preferable to ingest lactic acid bacteria at a dose of 5 billion to 200 billion (5×10^9 to 2×10^{11}) lactic acid bacteria per day from a viewpoint of effectiveness of

reducing the amount of mycotoxin. In addition, it is preferable to ingest a food composition containing lactic acid bacteria for at least six weeks from a viewpoint of effectiveness of reducing the amount of mycotoxin.

[0019]

The food composition according to the present invention contains lactic acid bacteria as an active ingredient. The lactic acid bacteria are preferably live cells from a viewpoint of exhibiting the effect of the present invention. Examples of lactic acid bacteria include: bacteria belonging to the genus *Lactocaseibacillus*, such as *Lactocaseibacillus paracasei*; bacteria belonging to the genus *Lactobacillus*, such as *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus mali*, *Lactobacillus helveticus*, *Lactobacillus gasseri*, *Lactobacillus fermentum*, *Lactobacillus salivarius*, *Lactobacillus yoghurtii*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactobacillus johnsonii*; bacteria belonging to the genus *Streptococcus*, such as *Streptococcus thermophilus*; bacteria belonging to the genus *Lactococcus*, such as *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus plantarum*, and *Lactococcus raffinolactis*; and bacteria belonging to the genus *Enterococcus*, such as *Enterococcus faecalis* and *Enterococcus faecium*.

[0020]

Among the above *Lacticaseibacillus paracasei*, *Lacticaseibacillus paracasei* YIT 9029 (FERM BP-1366, Entrusted Date: May 1, 1981) has been internationally deposited with National Institute of Technology and Evaluation Patent Organism Depositary (Room 120, Kazusakamatari 2-5-8, Kisarazu-shi, Chiba, Japan, 292-0818), which is an international depositary authority under the Budapest Treaty. Note that *Lacticaseibacillus paracasei* YIT 9029 was classified into *Lactobacillus casei* in the previous classification, but bacteria belonging to the genus *Lactobacillus* were recently reclassified, and *Lacticaseibacillus paracasei* YIT 9029 was classified into *Lacticaseibacillus paracasei* (Zheng et al., A taxonomic note on the genus *Lactobacillus* : Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *Int. J. Syst. Evol. Microbiol.* 2020 Apr; 70(4):2782-2858 DOI 10.1099/ijsem.0.004107). In the present specification, the classification of lactic acid bacteria is expressed by a new classification classified on the basis of the above document.

[0021]

Among these, lactic acid bacteria belonging to the genus *Lacticaseibacillus* are preferable, and

Lacticaseibacillus paracasei YIT 9029 (FERM BP-1366) is more preferable from a viewpoint of effectiveness of reducing the amount of mycotoxin.

[0022]

The food composition may be in any form as long as the amount of mycotoxin is effectively reduced, and may be any food and drink product, for example, in a liquid form, a semi-solid form, or a solid form. Specific examples thereof include a fermented food and drink product and a dried bacterial powder, and a food and drink product containing fermented milk is particularly preferable.

[0023]

The food composition may contain various carbohydrates and milk proteins in addition to the above lactic acid bacteria, and may further contain a dietary fiber, a flavor, and the like as necessary.

[0024]

The various carbohydrates may contain saccharides such as fructose, maltitol, glucose, lactose, sucrose, isomerized sugar, palatinose, trehalose, and xylose; sugar alcohols such as sorbitol, xylitol, erythritol, lactitol, palatinit, reduced starch syrup, and reduced maltose starch syrup; and a high-intensity sweetener such as aspartame, sucralose, stevia, or alitame.

[0025]

Examples of the milk protein include casein and whey protein. As for the milk protein, skim milk, whole milk powder, milk protein concentrate, whey protein concentrate, whey protein isolate, caseinate, and the like may be contained as those containing these components.

[0026]

The dietary fiber may contain polydextrose, dextrin, starch, pullulan, mannan, glucomannan, alginic acid, xanthan gum, guar gum, tamarind gum, chitin, chitosan, glycogen, inulin, pectin, agar, and the like.

[0027]

The flavor may contain a yogurt-based flavor, a berry-based flavor, a citrus-based flavor, a Chinese quince-based flavor, a perilla-based flavor, an apple-based flavor, a mint-based flavor, a grape-based flavor, an apricot-based flavor, and the like.

[0028]

In addition, an excipient, a stabilizer, a preservative, a wetting agent, an emulsifier, a lubricant, a sweetener, a coloring agent, a flavor, a buffering agent, an antioxidant, a pH adjusting agent, and the like may be appropriately added to the food composition as necessary.

[0029]

Regarding an ingestion amount of the food composition of the present invention, it is preferable to ingest lactic

acid bacteria at a dose of 5 billion to 200 billion lactic acid bacteria per day, it is more preferable to ingest lactic acid bacteria at a dose of 10 billion to 200 billion lactic acid bacteria per day, and it is particularly preferable to ingest lactic acid bacteria at a dose of 20 billion to 200 billion lactic acid bacteria per day from a viewpoint of effectiveness of reducing the amount of mycotoxin.

[0030]

When the dose is less than the above lower limit, there is a possibility that effectiveness of reducing the amount of mycotoxin cannot be confirmed. On the other hand, when the dose is more than the upper limit, there is a possibility that appearance of a product is poor, for example, precipitation occurs in a container in a case of a beverage.

[0031]

An ingestion period of the food composition of the present invention is preferably at least six weeks, more preferably at least ten weeks, and most preferably at least 12 weeks. When the ingestion period is less than the above lower limit, there is a possibility that effectiveness of reducing the amount of mycotoxin cannot be confirmed.

Examples

[0032]

(Examples)

Hereinafter, the present invention will be described specifically on the basis of the following examples, but the present invention is not limited thereto.

[0033]

(Example 1)

<Food and drink product for test>

As a test beverage, 80 mL of each of a lactic acid bacteria beverage and a placebo beverage was prepared in a bottle container. The placebo beverage has a taste, a color, an appearance, and nutrients similar to the lactic acid bacteria beverage, but does not contain lactic acid bacteria. Components of the lactic acid bacteria beverage and the placebo beverage are described below.

[0034]

<Components of lactic acid bacteria beverage>

- Fructose, maltitol, skim milk, polydextrose, glucose, live cells of *Lactobacillus paracasei* YIT 9029 (FERM BP-1366, 3×10^{10} CFU/80 mL), and flavor

<Components of placebo beverage>

- Fructose, maltitol, skim milk, polydextrose, glucose, and flavor

[0035]

(Example 2)

<Subject>

174 healthy adults of 20 to 60 years old in whom aflatoxin was detected in blood and in urine in a preliminary study were adopted as subjects. A test was performed on a total of 174 persons including 52 Malays, 96 Chinese, and 26 Indians. Of the 174 subjects, 87 subjects were assigned to an ingestion group of the lactic acid bacteria beverage (hereinafter, referred to as a lactic acid bacteria ingesting group), and 87 subjects were assigned to an ingestion group of the placebo beverage (hereinafter, referred to as a placebo ingestion group). After start of the test, each of the subjects ingested a test beverage twice per day for 12 weeks within one hour after lunch and after dinner. In addition, during a follow-up period after a lapse of 12 weeks, each of the subjects did not ingest the test beverage.

[0036]

(Example 3)

<Evaluation items>

A urine sample and a blood sample were collected from each of the subjects, and the amount of aflatoxin (AFM₁) in urine and the amount of aflatoxin (AFB₁) in blood were evaluated. In addition, a meal survey of the subjects was conducted, and a relationship to AFM₁ and AFB₁ was evaluated.

[0037]

<Test method>

A model of a generalized estimating equation (GEE) was set and used for statistical analysis in Examples of the present application. Statistical significance was determined by a known test method. Specifically, in order to compare continuous variables between the lactic acid bacteria ingestion group and the placebo ingestion group or to determine a baseline relationship between categorical variables, presence or absence of a significant difference between two or more groups was determined using Mann-Whitney U test or Kruskal-Wallis H test. For a relationship between continuous variables, Spearman's correlation was used as a correlation coefficient. A p value was used as an index of a test result, and it was defined that the test result had a p value of less than 0.05 in a statistically significant relationship. Note that, when the invention of the present application is performed, any method capable of determining statistical significance can be used without being limited to the above method.

[0038]

(Example 4)

<Test schedule>

Fig. 1 is a diagram for explaining a test schedule in

the present invention. The horizontal axis indicates a sampling time. The upper row indicates the lactic acid bacteria ingestion group, and the lower row indicates the placebo ingestion group.

[0039]

In each of the lactic acid bacteria ingestion group and the placebo ingestion group, " Δ " indicates a timing of urine collection, and " $\blacktriangle$ " indicates a timing of blood collection. Urine collection was performed nine times in total, and blood collection was performed five times in total. In addition, the ingestion of the lactic acid bacteria beverage and the placebo beverage was performed until 12 weeks elapsed from start of the ingestion. In addition, as for sampling, urine collection and blood collection were performed continuously as a follow-up period until four weeks elapsed after an end of the ingestion period.

[0040]

<Quantification of AFM₁>

Quantification of the amount of aflatoxin (AFM₁) in urine was performed using a commercially available kit (Aflatoxin M₁ ELISA Quantitative test kit, catalogue number 991AFLM01U-96, Helica Biosystems Inc.) by partially modifying the method of Mold et al. (Association between aflatoxin M₁ excreted in human urine samples with the

consumption of milk and dairy products. Bulletin of Environmental Contamination and Toxicology, 89(6), 1115-1119). Specifically, a urine sample was centrifuged at 3000xg for five minutes for precipitate removal. Thereafter, each of the supernatant and a urine standard substance (0 to 4 ng/mL) was diluted with distilled water at 1 : 20. The diluted standard or sample (100 μ L) was mixed with an assay buffer (200 μ L), and 100 μ L of the mixture was transferred to microwells pre-coated with an antibody. The microwells were incubated for one hour to bind AFM₁ in the standard or the sample to the antibody with which the wells were coated. Thereafter, the contents were decanted from the microwells and washed three times with a PBS-Tween wash buffer using a microplate washer. Approximately 100 μ L of conjugate was added, and the mixture was incubated for 15 minutes such that the remaining antibody with which the wells were coated was bound. Thereafter, decantation and washing were repeated three times, a substrate reagent (100 μ L) was added, and the mixture was incubated in a dark place at room temperature for 15 minutes. Thereafter, color development was immediately measured at an absorbance of 450 nm and compared with a standard curve to quantify AFM₁.

[0041]

<Quantification of AFB₁>

Quantification of the amount of aflatoxin (AFB₁) in blood was performed by the following method. First, serum was separated by the method of Mold et al. (Detection of serum AFB₁-lysine adduct in Malaysia and its association with liver and kidney functions. International Journal of Hygiene and Environmental Health, 217(4-5), 443-451), and 250 µL of the obtained serum sample was transferred to a capped vial, and cryopreserved at - 80°C until analysis. Thereafter, AFB₁ was quantified by high-performance liquid chromatography with fluorescence detection by the method of Andrews et al. (Andrews-Trevino, J. Y., Webb, P., Shively, G., Kablan, A., Baral, K., Davis, D., Paudel, K., Shrestha, R., Pokharel, A., & Acharya, S. (2021). Aflatoxin exposure and child nutrition: measuring anthropometric and long-bone growth over time in Nepal. The American journal of clinical nutrition, 113(4), 874-883).

[0042]

(Example 5)

<Amount of aflatoxin in urine>

Fig. 2 is a table for explaining the amount of aflatoxin in urine in Examples of the present invention. In the table, the first column indicates a sampling time, the second column indicates a geometric mean (ng/ml) of the AFM₁ amount in the lactic acid bacteria ingestion group, the third column indicates a geometric mean (ng/ml) of the

AFM₁ amount in the placebo ingestion group, and the fourth column indicates a p value calculated by Mann-Whitney U test/Kruskal-Wallis H test. Note that, in the following paragraphs, when a significant difference can be confirmed, a cell in the drawing is indicated in gray.

[0043]

From Fig. 2, it could be confirmed that the geometric mean of the AFM₁ amount in the lactic acid bacteria ingestion group was lower than that in the placebo ingestion group at all time points after start of the ingestion. This condition persisted not only during the ingestion period but also until the follow-up period after four weeks after an end of the ingestion.

In addition, it could be confirmed that a difference between the AFM₁ amount in the lactic acid bacteria ingestion group and the AFM₁ amount in the placebo ingestion group was larger after the sixth week of the ingestion than that up to the fourth week of the ingestion.

In addition, it could be confirmed that the AFM₁ amount in the lactic acid bacteria ingestion group continued to decrease as compared with that in the placebo ingestion group from the tenth week to the twelfth week of the ingestion, and the AFM₁ amount at the twelfth week of the ingestion significantly decreased.

[0044]

(Example 6)

<Amount of aflatoxin in blood>

Fig. 3 is a table for explaining the amount of aflatoxin in blood in Examples of the present invention. In the table, the first column indicates a sampling time, the second column indicates a geometric mean (pg/mg alb) of the AFB₁ amount in the lactic acid bacteria ingestion group, the third column indicates a geometric mean (pg/mg alb) of the AFB₁ amount in the placebo ingestion group, and the fourth column indicates a p value calculated by Mann-Whitney U test/Kruskal-Wallis H test. Note that since AFB₁ was detected as an AFB₁-lysine adduct, the unit is "pg/mg alb".

From Fig. 3, it could be confirmed that the geometric mean of the AFB₁ amount in the lactic acid bacteria ingestion group was lower than that in the placebo ingestion group at all time points after start of the ingestion. This condition persisted not only during the ingestion period but also until the follow-up period after four weeks after an end of the ingestion.

[0045]

(Example 7)

<Food characteristics>

Fig. 4 is a table presenting a relationship between the amount of aflatoxin in urine or in blood and a food

ingested by a subject within a sampling period in Examples of the present invention. In the table, the first column indicates the type of food, the second column indicates a correlation coefficient (ρ , ng/ml) and a p value of the amount of aflatoxin in urine (AFM₁ amount), and the third column indicates a correlation coefficient and a p value of the amount of aflatoxin in blood (AFB₁ amount). As the correlation coefficient, Spearman's correlation was used. Since the AFB₁ amount was detected as an AFB₁-lysine adduct amount, the unit is "pg/mg alb". From Fig. 4, it was found that there was a significant correlation between ingestion of cereals and an increase in the AFM₁ amount, but there was no significant correlation between ingestion of protein (meat, fish, beans, eggs, and dairy products) and the AFM₁ amount. Note that a case where the correlation coefficient is negative indicates that the amount of aflatoxin in a body has decreased due to ingestion of a corresponding type of food.

[0046]

(Conclusion)

From the above, the following points were clarified.

- From Fig. 2, it could be confirmed that the geometric mean of the AFM₁ amount (that is, the amount of aflatoxin in urine) in the lactic acid bacteria ingestion group was lower than that in the placebo ingestion group at

all time points after start of the ingestion. This condition persisted not only during the ingestion period but also until the follow-up period after four weeks after an end of the ingestion. In addition, it could be confirmed that a difference between the AFB₁ amount in the lactic acid bacteria ingestion group and the AFB₁ amount in the placebo ingestion group was larger after the sixth week of the ingestion than that up to the fourth week of the ingestion. In addition, it could be confirmed that the AFB₁ amount in the lactic acid bacteria ingestion group continued to decrease as compared with that in the placebo ingestion group from the tenth week to the twelfth week of the ingestion, and significantly decreased at a time point of the twelfth week of the ingestion.

- From Fig. 3, it could be confirmed that the geometric mean of the AFB₁ amount in the lactic acid bacteria ingestion group was lower than that in the placebo ingestion group at all time points after start of the ingestion. This condition persisted not only during the ingestion period but also until the follow-up period after four weeks after an end of the ingestion.

- In Fig. 4, it was clarified that cereals are susceptible to aflatoxin contamination. Therefore, it can be said that a subject having a lifestyle of ingesting cereals is more likely to receive the effect of the food

composition of the present application of reducing the amount of mycotoxin absorbed into a body.

CLAIMS

[Claim 1]. A food composition comprising lactic acid bacteria as an active ingredient, wherein the food composition reduces an amount of mycotoxin absorbed into a body.

[Claim 2]. The food composition according to claim 1, wherein the mycotoxin is aflatoxin.

[Claim 3]. The food composition according to claim 2, wherein the food composition reduces the amount of aflatoxin in blood or in urine in the body.

[Claim 4]. The food composition according to claim 3, wherein the lactic acid bacteria are administered to a subject at a dose of 5×10^9 to 2×10^{11} cells per day for at least six weeks.

[Claim 5]. The food composition according to claim 3, wherein aflatoxin detected in urine is aflatoxin M₁, and aflatoxin detected in blood is aflatoxin B₁.

[Claim 6]. The food composition according to claim 3, wherein the subject is a human or livestock.

[Claim 7]. The food composition according to any one of claims 1 to 6, wherein the lactic acid bacteria are administered for at least ten weeks.

[Claim 8]. The food composition according to any one of claims 1 to 6, wherein the lactic acid bacteria are administered for at least 12 weeks.

[Claim 9]. The food composition according to any one of claims 1 to 6, wherein the food composition is a beverage further comprising a carbohydrate and a milk protein.

[Claim 10]. The food composition according to any one of claims 1 to 6, wherein the lactic acid bacteria belong to the genus *Lacticaseibacillus*.

[Claim 11]. The food composition according to claim 10, wherein the lactic acid bacteria are *Lacticaseibacillus paracasei* YIT 9029 (FERM BP-1366).

[Claim 12]. A mycotoxin-reducing agent comprising *Lacticaseibacillus paracasei* as an active ingredient, wherein the agent reduces an amount of mycotoxin absorbed into a body.

[Claim 13]. The mycotoxin-reducing agent according to claim 12, wherein the *Lacticaseibacillus paracasei* is *Lacticaseibacillus paracasei* YIT 9029 (FERM BP-1366).