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- (56) Related Art
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(54) **Title:** NOVEL BIFIDOBACTERIUM GENUS BACTERIUM HAVING HIGH CAPACITY TO UTILIZE
POLYSACCHARIDES OF DIETARY ORIGIN

(54) 発明の名称: 食事由来の多糖の利用性が高い新規ビフィドバクテリウム属細菌

(57) **Abstract:** Provided is a novel Bifidobacterium strain that can utilize xylans. A bacterium of the genus Bifidobacterium
including a xylanase gene in the genome thereof.

(57) 要約: キシラン類を利用可能な新規ビフィズス菌株を提供する。キシラナーゼ遺
伝子をゲノム上に有するビフィドバクテリウム属細菌。



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Description

NOVEL BIFIDOBACTERIUM GENUS BACTERIUM HAVING HIGH
CAPACITY TO UTILIZE POLYSACCHARIDES OF DIETARY ORIGIN

Technical Field

[0001]

The present invention relates to bacteria belonging to the genus *Bifidobacterium* classified as *Bifidobacterium pseudocatenulatum*.

Background Art

[0002]

One of the causes of influence of probiotics on the physiological function of a host is presumed to be that probiotics metabolize carbohydrates sources remaining in the digestive tract and produce short-chain fatty acids such as lactic acid and acetic acid. Previous studies reveal that the genomes of bifidobacterial species dominant in the intestinal tracts of adults have more genes involved in metabolism of plant-derived indigestible polysaccharides, particularly xylans contained in a large amount in diets for adults, in comparison to the genomes of infant-derived bifidobacterial species.

[0003]

Thus, *Bifidobacterium* strains having a high capacity to utilize indigestible polysaccharides are expected to

grow in the intestines of adults and have a high organic acid producing ability, and therefore may become a promising candidate for probiotics for adults.

[0004]

It has been heretofore known that as bifidobacteria capable of utilizing indigestible polysaccharides, for example, *Bifidobacterium breve* strains (Non Patent Literature 1) and *Bifidobacterium bifidum* strains (Non Patent Literature 2) capable of utilizing starch, and *Bifidobacterium pseudocatenulatum* strains (Non Patent Literature 3) capable of utilizing arabinogalactan are present.

[0005]

However, bifidobacteria capable of cleaving the xylose backbones of xylans which are typical indigestible polysaccharides contained in the diets have not heretofore been known.

Citation List

Non Patent Literature

[0006]

Non Patent Literature 1: BMC Genomics (2014) 15:170

Non Patent Literature 2: Environmental Microbiology (2015) 17(7), 2515-2531

Non Patent Literature 3: Japanese Journal of Food Microbiology, Vol.24 No.4 Page.163-170

Summary of Invention

Technical Problem

[0007]

The present invention relates to provision of a novel *Bifidobacterium* strain capable of utilizing xylans.

Solution to Problem

[0008]

The present inventors extensively conducted studies, and resultantly isolated and identified a novel microbial strain capable of efficiently utilizing xylans from the human intestine.

[0009]

Specifically, the present invention relates to the following 1) to 7).

1) Bacteria belonging to the genus *Bifidobacterium* having a xylanase gene on a genome, wherein the xylanase gene is a polynucleotide consisting of the nucleotide sequence set forth as SEQ ID NO: 1, or a polynucleotide having an identity of 70% or more with the nucleotide sequence and encoding a protein having xylanase activity, and wherein the bacteria have an ability to utilize xylans.

2) The bacteria belonging to the genus *Bifidobacterium* according to 1), wherein the xylans are xylan and arabinoxylan.

3) The bacteria belonging to the genus *Bifidobacterium* according to 1) or 2), which have an ability to utilize starch.

4) The bacteria belonging to the genus *Bifidobacterium* according to any of 1) to 3), wherein the bacteria belonging to the genus *Bifidobacterium* are classified as *Bifidobacterium pseudocatenulatum*.

5) *Bifidobacterium pseudocatenulatum* YIT11027 (NITE BP-02928), *Bifidobacterium pseudocatenulatum* YIT11055 (NITE BP-02929), *Bifidobacterium pseudocatenulatum* YIT11057 (NITE BP-02930), *Bifidobacterium pseudocatenulatum* YIT11952 (NITE BP-02931), *Bifidobacterium pseudocatenulatum* YIT11954 (NITE BP-02932), or *Bifidobacterium pseudocatenulatum* YIT12989 (NITE BP-02933).

6) A food or beverage comprising the bacteria belonging to the genus *Bifidobacterium* according to any of 1) to 5).

7) The food or beverage according to 6), which is a fermented milk food or beverage.

Advantageous Effects of Invention

[0010]

The novel bacteria belonging to the genus *Bifidobacterium* according to the present invention have an ability to degrade and utilize xylans contained in a large amount in diets for adults, in particular, arabinoxylan. Thus, the *Bifidobacterium*

pseudocatenulatum of the present invention is expected to grow in the intestines of adults and have a high organic acid producing ability, and can be used for medicinal products, food and the like as probiotics for adults.

Brief Description of Drawings

[0011]

[Figure 1] Figure 1 shows the xylose backbone cleaving activity of the YIT11057 strain.

[Figure 2] Figure 2 shows the acid/bile acid resistance of the YIT11057 strain.

Description of Embodiments

[0012]

The type of the bacteria belonging to the genus *Bifidobacterium* of the present invention is not particularly limited as long as the bacteria have a xylanase gene on a genome. The type of the bacteria belonging to the genus *Bifidobacterium* may be any of, for example, *Bifidobacterium pseudocatenulatum*, *Bifidobacterium breve*, *Bifidobacterium longum* (*B. longum*), *Bifidobacterium bifidum* (*B. bifidum*), *Bifidobacterium animalis* (*B. animalis*), *Bifidobacterium suis* (*B. suis*), *Bifidobacterium infantis* (*B. infantis*), *Bifidobacterium adolescentis* (*B. adolescentis*), *Bifidobacterium catenulatum* (*B. catenulatum*), *Bifidobacterium lactis* (*B. lactis*), *Bifidobacterium globosum* (*B. globosum*), *Bifidobacterium angulatum* (*B.*

angulatum) and *Bifidobacterium dentium* (*B. dentium*), and is preferably *Bifidobacterium pseudocatenulatum*.

Bifidobacteria having a xylanase gene on the genome have not heretofore been known, and the bacteria belonging to the genus *Bifidobacterium* of the present invention are novel bacteria.

[0013]

The term "xylanase gene" refers to a gene encoding xylanase classified as GH10 (endo-1,4-beta-xylanase A; xynA), and the "xylanase gene" held by the bacteria belonging to the genus *Bifidobacterium* of the present invention on a genome thereof specifically includes a polynucleotide consisting of the nucleotide sequence set forth as SEQ ID NO: 1 or a polynucleotide having an identity of 70% or more, preferably 90% or more, more preferably 95% or more, still more preferably 98% or more, still more preferably 99% or more with the nucleotide sequence and encoding a protein having xylanase activity.

Here, the polynucleotide consisting of the nucleotide sequence set forth as SEQ ID NO: 1 refers to a xylanase gene held by the later-described YIT11057 strain.

[0014]

The identity of the nucleotide sequence is a value obtained by aligning two nucleotide sequences to be compared in such a manner that the two nucleic acid sequences are in match with each other as much as

possible, dividing the number of matched bases by the total number of bases, and expressing the quotient in percentage. Those skilled in the art can appropriately set parameters of software such as BLAST, ClustalX or Genetyx to determine the identity of the nucleotide sequences.

The term "xylanase activity" means activity of hydrolyzing the xylose backbones of xylans using the xylans as a substrate to produce xylooligosaccharides and xylose (xylan hydrolyzing activity).

Here, examples of the xylans include xylan, arabinoxylan, glucuronoxytan, glucuronoarabinoxylan and acetylxylan, and xylan and arabinoxylan are preferable.

Thus, in other words, the bacteria belonging to the genus *Bifidobacterium* having a xylanase gene on a genome in the present invention are bacteria belonging to the genus *Bifidobacterium* having an ability to cleave the xylose backbones of xylans.

[0015]

In the present invention, the phrase "having an ability to utilize xylans" means having an ability to grow with xylans as carbon sources. Specifically, the phrase means that, for example, it is possible to grow in a medium containing only xylans as carbon sources. For example, the phrase means that when culturing is performed for 72 hours in a medium containing xylans as the only carbohydrate source, the turbidity is, for example, 0.1 or more in terms of increase in OD₆₀₀. The

turbidity is preferably 0.3 or more, more preferably 0.4 or more in terms of increase in OD₆₀₀. When the change of turbidity cannot be measured due to the medium containing xylans having a large amount of insoluble fractions or for other reasons, the phrase means that the amount of short-chain fatty acids produced in the culture supernatant after 72-hour culturing, for example, the total amount of lactic acid, acetic acid and formic acid produced, is 10 mM or more. The amount of short-chain fatty acids produced is preferably 20 mM or more, more preferably 40 mM or more in total.

[0016]

The medium to be used in a test on the xylans utilizing ability may be one obtained by adding xylans to a medium free of carbohydrate sources, and the composition of the medium free of carbohydrate sources can be the composition of M-ILS liquid medium, Peptone-Yeast (PY) medium or the like.

The amount of xylans added to the medium is preferably 0.01 to 10 mass%, more preferably 0.05 to 5 mass%, still more preferably 0.1 to 1 mass% in the medium. It is desirable that as a control, bacteria belonging to the genus *Bifidobacterium* of interest be cultured in a medium free of carbohydrate sources, followed by checking OD values.

[0017]

As described above, the bacteria belonging to the genus *Bifidobacterium* having a xylanase gene on a genome

in the present invention have an ability to degrade and utilize xylans, and therefore can contribute to supply of short-chain fatty acids such as acetic acid, lactic acid and formic acid produced by degradation of xylans.

[0018]

The bacteria belonging to the genus *Bifidobacterium* of the present invention can be obtained by screening from bacteria belonging to the genus *Bifidobacterium* present in, for example, the intestines of humans (e.g. adults or infants), preferably *Bifidobacterium pseudocatenulatum*, using the presence of a xylanase gene or the above ability to utilize xylans as an indicator. For example, from *Bifidobacterium pseudocatenulatum*, 16 strains shown in Examples below, and further six strains of YIT11027, YIT11055, YIT11057, YIT11952, YIT11954 and YIT12989 can be selected. The bacteria belonging to the genus *Bifidobacterium* of the present invention are not limited thereto and include bacterial strains which are biologically and genetically closely related thereto, as long as the bacteria belonging to the genus *Bifidobacterium* have a xylanase gene on a genome. The bacteria belonging to the genus *Bifidobacterium* of the present invention may be a natural strain present in nature, a variant of a natural strain, or a genetically modified species as long as they have a xylanase gene on a genome.

[0019]

As shown below, YIT11027 strain, YIT11055 strain, YIT11057 strain, YIT11952 strain, YIT11954 strain and YIT12989 strain were deposited at the National Institute of Technology and Evaluation, Patent Microorganisms Depositary (#122, Kazusakamatari 2-5-8, Kisarazu-shi, Chiba) on March 25, 2019.

Bifidobacterium pseudocatenulatum YIT11027 (NITE BP-02928),

Bifidobacterium pseudocatenulatum YIT11055 (NITE BP-02929),

Bifidobacterium pseudocatenulatum YIT11057 (NITE BP-02930),

Bifidobacterium pseudocatenulatum YIT11952 (NITE BP-02931),

Bifidobacterium pseudocatenulatum YIT11954 (NITE BP-02932), and

Bifidobacterium pseudocatenulatum YIT12989 (NITE BP-02933).

Of these, the YIT11027 strain, YIT11057 strain, YIT11954 strain or bacterial strains closely related thereto are preferable from the viewpoint of an ability to utilize xylans and further from the viewpoint that they also have a starch-utilization ability in combination. Further, YIT11057 strain or bacterial strains closely related thereto are more preferable from the viewpoint that they have acid/bile acid resistance and also have the properties that no lysogenic phage is detected in the genome.

Here, the term "starch-utilization ability" means having an ability to grow with starch as carbon sources. The term "acid/bile acid resistance" means the viability after continuous exposure to artificial gastric acid and artificial bile acid. The term "no lysogenic phage" means that a region having high homology with an existing phage has not been detected in a genome sequence by use of PHASTER which is a phage search program server (<http://phaster.ca/>).

[0020]

The term "closely related bacterial strains" indicates bacterial strains having identical sequences in Multilocus sequence analysis (MLSA) for evaluating a plurality of housekeeping gene sequences.

Specifically, the full-length sequences of DNA gyrase subunit B (gyrB), 50S ribosomal protein L2 (rplB), Amidophosphoribosyltransferase (purF), DNA-directed RNA polymerase subunit beta (rpoB), ATP-dependent Clp protease ATP-binding subunit ClpC1 (clpC), Elongation factor G (fusA) and Isoleucine-tRNA ligase (ileS) reported as genes suitable for MLSA analysis of bifidobacteria (International Journal of Systematic and Evolutionary Microbiology (2006), 56, 2783-2792) are acquired, all genes are linked for each bacterial strain, and the coincidence in sequence between strains for comparison is then examined using a program such as VSEARCH or BLAST. When the sequence identity is 90% or more, preferably 95% or more, more preferably 98% or

more, still more preferably 99% or more, it can be determined that the bacterial strains are closely related species.

The nucleotide sequences of *gyrB*, *rplB*, *purF*, *rpoB*, *clpC*, *fusA* and *ileS* genes in YIT11057 strain are shown in the sequence listing (*gyrB*: SEQ ID NO: 2, *rplB*: SEQ ID NO: 3, *purF*: SEQ ID NO: 4, *rpoB*: SEQ ID NO: 5, *clpC*: SEQ ID NO: 6, *fusA*: SEQ ID NO: 7, and *ileS*: SEQ ID NO: 8).
[0021]

The application form of the bacteria belonging to the genus *Bifidobacterium* of the present invention is not particularly limited, and may be lyophilized bacteria, or a cultured product containing these bacteria can be utilized. In any of the forms, the bacteria are preferably in a viable state.
[0022]

The bacteria belonging to the genus *Bifidobacterium* of the present invention can also be mixed with a solid or liquid pharmaceutical nontoxic carrier and utilized in the form of a common pharmaceutical preparation. Examples of the preparation include solid preparations such as tablets, granules, powders and capsules, liquid preparations such as solutions, suspensions and emulsions, and lyophilized preparations. These preparations can be prepared by conventional means for production of preparations. Examples of the pharmaceutical nontoxic carrier include glucose, lactose, sucrose, starch, mannitol, dextrin, fatty acid

glycerides, polyethylene glycol, hydroxyethyl starch, ethylene glycol, polyoxyethylene sorbitan fatty acid esters, amino acids, gelatin, albumin, water and physiological saline. If necessary, common additives such as stabilizers, wetting agents, emulsifiers, binders, tonicity agents and excipients can also be appropriately added.

[0023]

The bacteria belonging to the genus *Bifidobacterium* of the present invention can be not only prepared into the above-described preparations but also added to a food or beverage for use. When added to a food or beverage, the bacteria belonging to the genus *Bifidobacterium* may be incorporated directly or together with various nutrient ingredients. Specifically, when added to a food or beverage, the bacterial belonging to the genus *Bifidobacterium* of the present invention may be made into a form suitable for eating, i.e. a granular form, a particulate form, a tablet, a capsule, a paste or the like using common means with appropriate use of additives usable for food or beverage, may be added to various foods, e.g. processed meat products such as ham and sausage, processed seafood products such as cooked minced fish (*kamaboko*) and fish sausage (*chikuwa*), bread, confectionary, butter and dry milk, or may be added to beverages such as water, fruit juice, milk, soft drinks and tea drinks. The food or beverage includes animal feeds.

[0024]

Further, as the food or beverage, fermented milk food or beverage and fermented food or beverage such as fermented soybean milk, fermented fruit juice and fermented vegetable juice which contain the bacteria belonging to the genus *Bifidobacterium* of the present invention in a viable state are suitably used, and in particular, the fermented milk food or beverage is preferably used. The fermented milk food or beverage may be produced in accordance with a conventional method, and for example, when fermented milk is produced, the bacteria belonging to the genus *Bifidobacterium* of the present invention is inoculated and cultured in a sterilized milk medium alone or together with other microorganisms, and the cultured product is subjected to homogenization treatment to obtain a fermented milk base. Next, a separately prepared syrup solution is added and mixed, the mixture is homogenized with a homogenizer or the like, and a flavor is further added to make a final product. The fermented milk food or beverage thus obtained can also be a product in any form such as a plain type free of syrup (sweetener), a soft type, a fruit flavor type, a solid form or a liquid form.

[0025]

To such fermented milk food or beverage, optional ingredients including sweeteners such as syrup, emulsifiers, thickeners (stabilizers) and various vitamins can be added. The following may be added: as

the syrup, saccharides such as glucose, sucrose, fructose, high-fructose corn syrup, glucose syrup, palatinose, trehalose, lactose, xylose, galactooligosaccharide (GOS), xylooligosaccharide (XOS), arabinoxylooligosaccharide (AXOS), xylan, arabinoxylan, arabinooligosaccharide (AOS), arabinan, maltose, honey and molasses; sugar alcohols such as sorbitol, xylitol, erythritol, lactitol, palatinit, reduced sugar syrup and reduced maltose syrup; and high intensity sweeteners such as aspartame, thaumatin, sucralose, acesulfame K and stevia; emulsifiers such as glycerin fatty acid esters, polyglycerin fatty acid esters, sorbitan fatty acid esters and lecithin; and thickeners (stabilizers) such as agar, gelatin, carrageenan, guar gum, xanthane gum, pectin, locust bean gum, gellan gum, carboxymethyl cellulose, soybean polysaccharide and propylene glycol alginate. In addition, the following may be added: vitamins such as vitamin A, vitamin B, vitamin C and vitamin E; minerals such as calcium, magnesium, zinc, iron and manganese; acidifiers such as citric acid, lactic acid, acetic acid, malic acid, tartaric acid and gluconic acid; milkfats such as cream, butter and sour cream; flavors based on yogurt, berry, orange, Chinese quince, perilla, citrus, apple, mint, grape, apricot, pear, custard cream, peach, melon, banana, tropical, herb, black tea, coffee and the like; herb extracts, brown sugar extracts and the like.

[0026]

For production of the fermented milk food or beverage, microorganisms other than the bacteria belonging to the genus *Bifidobacterium* of the present invention can also be used in combination. Examples of such microorganisms include bacteria belonging to the genus *Lactobacillus* such as *Lactobacillus casei*, *Lactobacillus acidophilus* (*L. acidophilus*), *Lactobacillus plantarum* (*L. plantarum*), *Lactobacillus buchneri* (*L. buchneri*), *Lactobacillus gallinarum* (*L. gallinarum*), *Lactobacillus amylovorus* (*L. amylovorus*), *Lactobacillus brevis* (*L. brevis*), *Lactobacillus rhamnosus* (*L. rhamnosus*), *Lactobacillus kefir* (*L. kefir*), *Lactobacillus paracasei* (*L. paracasei*), *Lactobacillus crispatus* (*L. crispatus*), *Lactobacillus zeae* (*L. zeae*), *Lactobacillus helveticus* (*L. helveticus*), *Lactobacillus salivarius* (*L. salivarius*), *Lactobacillus gasseri* (*L. gasseri*), *Lactobacillus fermentum* (*L. fermentum*), *Lactobacillus reuteri* (*L. reuteri*), *Lactobacillus delbrueckii* subspecies. *bulgaricus* (*L. delbrueckii* subsp. *bulgaricus*), *Lactobacillus delbrueckii* subspecies. *delbrueckii* (*L. delbrueckii* subsp. *delbrueckii*) and *Lactobacillus johnsonii* (*L. johnsonii*), bacteria belonging to the genus *Streptococcus* such as *Streptococcus thermophilus*, bacteria belonging to the genus *Lactococcus* such as *Lactococcus lactis* subspecies. *lactis* (*Lactococcus lactis* subsp. *lactis*), *Lactococcus lactis* subspecies. *cremoris* (*Lactococcus lactis* subsp. *cremoris*), bacteria belonging to the genus *Enterococcus*

such as *Enterococcus faecalis* and *Enterococcus faecium* (*E. faecium*), bacteria belonging to the genus *Bacillus* such as *Bacillus subtilis*, and yeasts belonging to *Saccharomyces* genus, *Torulaspora* genus and *Candida* genus such as *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* and *Candida kefyr*. It is preferable that one or more selected from the group consisting of bacteria belonging to the genus *Lactobacillus*, *Streptococcus* and *Lactococcus* be used in combination with the bacteria belonging to the genus *Bifidobacterium* of the present invention to produce fermented milk food or beverage because a highly pleasant taste can be obtained, so that eating or drinking is facilitated.

[0027]

When the bacteria belonging to the genus *Bifidobacterium* of the present invention is used, the dose thereof is not strictly limited, and the suitable dose thereof is preferably 10^5 cfu to 10^{13} cfu, particularly preferably 10^8 cfu to 10^{12} cfu a day in terms of the number of viable bacteria.

Examples

[0028]

Example 1: Screening of bacteria belonging to the genus *Bifidobacterium* having an ability to utilize xylans

i) Bacterial strains used

53 Strains isolated from human feces and identified as *Bifidobacterium pseudocatenulatum* were screening targets.

[0029]

ii) Substrate

Xylan (derived from oat), arabinoxylan (derived from wheat) and xylooligo-saccharide (XOS) industrially produced by enzymatically treating xylan were used for analysis. For xylan and arabinoxylan, the results of examination by HPLC did not show the presence of monosaccharide (data is not shown). In addition, xylose and arabinose which are constituent monosaccharides of xylan and arabinoxylan, respectively, were analyzed, and as controls for confirmation of growth, starch which is a typical indigestible polysaccharide and glucose which is a constituent monosaccharide of starch, and lactose were also analyzed. After a 1% suspension of each substrate was prepared, xylan, arabinoxylan and starch were subjected to autoclave sterilization, and other substrates were subjected to sterilization by filtration using a 0.22 μm filter.

[0030]

iii) Test on utilization properties

For preventing suppression of growth of bacteria due to a growth-associated decrease in pH, an m-PY medium containing 100 mM PIPES was used. The 1% substrate solution was mixed with an equal amount of two-fold concentrated m-PY medium stored under refrigeration in a

nitrogen-filled atmosphere until use, and the mixture was used for a culture test (final concentration: 0.5%).

The frozen bacterial liquid was inoculated in a modified GAM agar, and then cultured for 2 to 3 days, and the thus-obtained colonies were subcultured in a modified GAM liquid medium containing 1% glucose/lactose. In the subculture, care was taken so as to scrape a large number of colonies. Stationary culturing was performed overnight at 37°C, and next morning, the bacterial liquid in an amount of 5% was subcultured again in a fresh one of the same medium. The bacterial liquid was cultured for about 6 to 9 hours until reaching the logarithmic growth phase, and the turbidity was then measured. 100 μ l of the culture solution was centrifuged, the supernatant was removed, and a carbohydrate source-free m-PY medium was then added to provide an OD₆₀₀ of 0.2 to suspend pellets. 198 μ l of each medium was dispensed to a 96-well plate, 2 μ l of the bacterial liquid was added, then 50 μ l of mineral oil was overlaid, and the bacteria were cultured at 37°C in a microplate reader PowerWave 340 (BIOTEC Co., Ltd.).

The turbidity was measured every 30 minutes, and the turbidity was monitored for 60 hours after the start of culturing. The above-described culturing operations were all carried out in an anaerobic glove box. After 60 hours, a portion of the culture solution was taken, deproteinized, and then subjected to HPLC to measure the amount of short-chain fatty acids produced. When a large

amount of insoluble particles were present in an m-PY medium containing xylan, the utilization properties were determined based on existence or non-existence of production of short-chain fatty acids because it was impossible to confirm growth of bacterial cells only by the measurement of turbidity. The short-chain fatty acids in the culture supernatant after culturing for 60 hours were measured by HPLC to confirm that the total amount of lactic acid, acetic acid and formic acid produced was 10 mM or more. Table 1 shows the number of strains which were capable of utilizing each of various substrates.

[0031]

[Table 1]

	Xylan	Arabinoxylan	XOS	Xylose	Arabinose	Starch	Glucose	Lactose
Number of utilizing strains	16	16	53	39	36	28	51	53
Number of non-utilizing strains	37	37	0	14	17	25	2	0

[0032]

A capacity to utilize xylooligosaccharides was shown in all bacterial strains of *B. pseudocatenulatum*, however, a capacity to utilize xylans having a longer chain varied among bacterial strains. 16 Strains which had utilized xylan and arabinoxylan were screened as bacteria belonging to the genus *Bifidobacterium* having an ability to utilize xylans.

[0033]

The bacterial strains belonging to the genus *Bifidobacterium* having an ability to utilize xylans were cultured in a liquid medium with xylan as the only carbohydrate source, and the oligosaccharides in the culture supernatant in the earlier logarithmic growth phase were analyzed by HPLC. The result showed that xylose and xylooligosaccharides had been produced. This revealed that the present bacteria had activity of cleaving the xylose backbone into xylose and xylooligosaccharides. Figure 1 shows, as a typical example, the results of analysis using YIT11057 strain which is one the 16 strains. Symbols X1 to X6 in Figure 1 denote xylose and xylooligosaccharides having corresponding numbers of xylose backbones, and it can be seen that xylose and xylooligosaccharides which had not been present in the medium were produced by culturing YIT11057 strain.

[0034]

Example 2: Properties of six strains of YIT11027, YIT11055, YIT11057, YIT11952, YIT11954 and YIT12989

The utilization properties of the six strains of YIT11027, YIT11055, YIT11057, YIT11952, YIT11954 and YIT12989 selected from the above 16 strains having an ability to utilize xylans are shown below. For the three bacterial strains of YIT11027, YIT11057 and YIT11954, it was possible to confirm the starch-utilization properties as well as the xylan-utilization properties (Table 2).

[0035]

[Table 2]

	Xylan	Arabinoxyln	XOS	Xylose	Arabinose	Starch	Glucose	Lactose
YIT 11027	+	+	+	+	+	+	+	+
YIT 11055	+	+	+	+	+	-	+	+
YIT 11057	+	+	+	-	-	+	+	+
YIT 11952	+	+	+	+	+	-	+	+
YIT 11954	+	+	+	+	+	+	+	+
YIT 12989	+	+	+	+	+	-	+	+
YIT 4037 ^T (type strain)	-	-	+	+	+	+	+	+

+: utilization properties found
-: non-utilization properties

[0036]

Example 3: Genomic analysis

Genomic DNA was extracted from the bacterial cells, and a draft genome was determined using a next-generation sequencer Miseq (Illumina, Inc.). The gene region was predicted, and then the carbohydrate-active enzyme gene database dbCAN was consulted for all amino acid sequences to extract carbohydrate-active enzyme genes. The result of searching for genes identical in capacity to utilize xylans and distribution showed that all of the strains having endo-1,4-beta-xylanase A gene (xynA) belonging to GH10 were capable of utilizing xylans. Any of the strains which do not have this gene was not capable of utilizing xylans.

[0037]

Example 4: Properties of YIT11057

For YIT11057, acid/bile acid resistance, exhaustive searching for pathogenic factors and detection of a lysogenic phage in the genome were evaluated.

1) Acid/bile acid resistance

i) Artificial juice composition

<Artificial gastric juice composition (pH 3.6)>

Pepsin 40 mg/L

Proteose peptone 5 g/L

Gastric mucin 1.5 g/L

NaCl 5 g/L

NaHCO₃ 3 g/L

KH₂PO₄ 1 g/L

3.6 N HCl adjusting pH to 3.6

<Artificial intestinal juice>

NaCl 5 g/L

KCl 1 g/L

NaHCO₃ 3 g/L

3 M Na₂CO₃ adjusting pH to 8.0

[0038]

ii) 0.1 ml of a bacterial liquid cultured overnight in an mGAM liquid medium containing 1% glucose/lactose was added to 2 ml of artificial gastric juice, and the mixture was incubated at 37°C for 1 hour. Thereafter, 5.5 ml of artificial intestinal juice and 0.5 ml of 16% bile acid were added, and the mixture was left to stand at 37°C for 1 hour. Before and after acid treatment and after bile acid treatment, the bacterial liquid was collected, and diluted with physiological saline, 50 µl of the diluted liquid was then applied to an mGAM agar, and CFU was measured. The result showed that the viability rate was about 0.1% even after bile acid treatment (Figure 2). Since CFU of bacteria without resistance after each of the treatments was equal to or less than the detection lower limit, YIT11057 was shown to have relatively high acid/bile acid resistance.

[0039]

2) Exhaustive searching for pathogenic factors

Draft genome information was input in the PATRIC server (<https://www.patricbrc.org/>) (Wattam et al., 2017), and existence or non-existence of genes having

high homology with sequences in the Virulence Factor database was examined. As a result, a pathogenic factor gene was not detected.

[0040]

3) Detection of lysogenic phages in genome

For genome information of bacterial strains, an attempt was made to detect lysogenic phages registered in the phage database using the PHASTER server (<http://phaster.ca/>) (Arndt et al., 2016). As a result, a lysogenic phage was not detected.

[0041]

Where any or all of the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification (including the claims) they are to be interpreted as specifying the presence of the stated features, integers, steps or components, but not precluding the presence of one or more other features, integers, steps or components.

[0042]

A reference herein to a patent document or any other matter identified as prior art, is not to be taken as an admission that the document or other matter was known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

The claims defining the invention are as follows:

[Claim 1]

Bacteria belonging to the genus *Bifidobacterium* having a xylanase gene on a genome, wherein the xylanase gene is a polynucleotide consisting of a nucleotide sequence set forth as SEQ ID NO: 1, or a polynucleotide having an identity of 90% or more with the nucleotide sequence and encoding a protein having xylanase activity, and wherein the bacteria have an ability to utilize xylans.

[Claim 2]

The bacteria belonging to the genus *Bifidobacterium* according to claim 1, wherein the xylans are xylan and arabinoxylan.

[Claim 3]

The bacteria belonging to the genus *Bifidobacterium* according to claim 1 or claim 2, which have an ability to utilize starch.

[Claim 4]

The bacteria belonging to the genus *Bifidobacterium* according to any one of claims 1 to 3, wherein the bacteria belonging to the genus *Bifidobacterium* are classified as *Bifidobacterium pseudocatenulatum*.

[Claim 5]

Bifidobacterium pseudocatenulatum YIT11027 (NITE BP-02928), *Bifidobacterium pseudocatenulatum* YIT11055 (NITE BP-02929), *Bifidobacterium pseudocatenulatum* YIT11057 (NITE BP-02930), *Bifidobacterium pseudocatenulatum* YIT11952 (NITE BP-02931), *Bifidobacterium pseudocatenulatum* YIT11954 (NITE BP-02932), or *Bifidobacterium pseudocatenulatum* YIT12989 (NITE BP-02933).

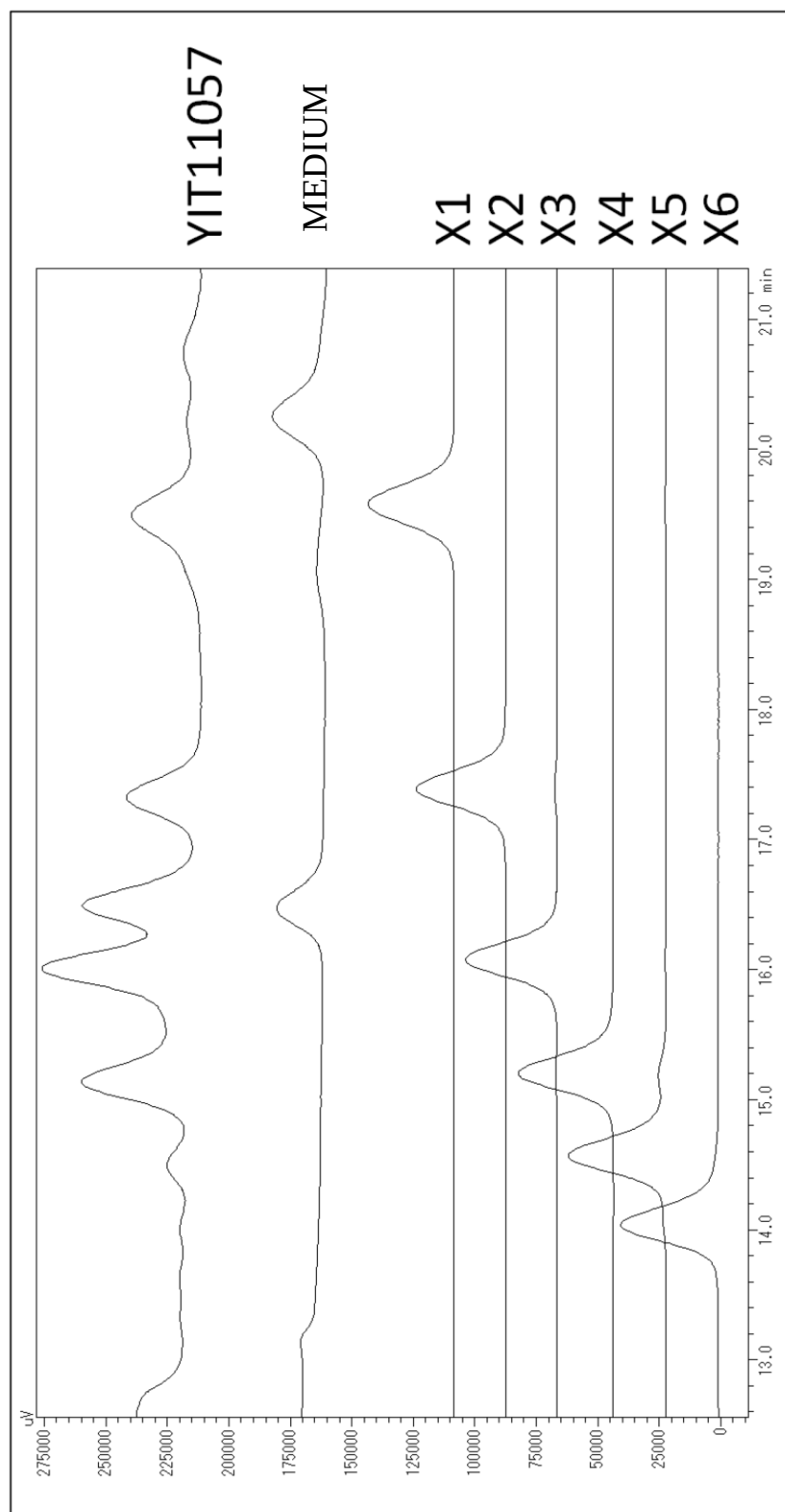
[Claim 6]

A food or beverage comprising the *Bifidobacterium* genus bacterium according to any one of claims 1 to 5.

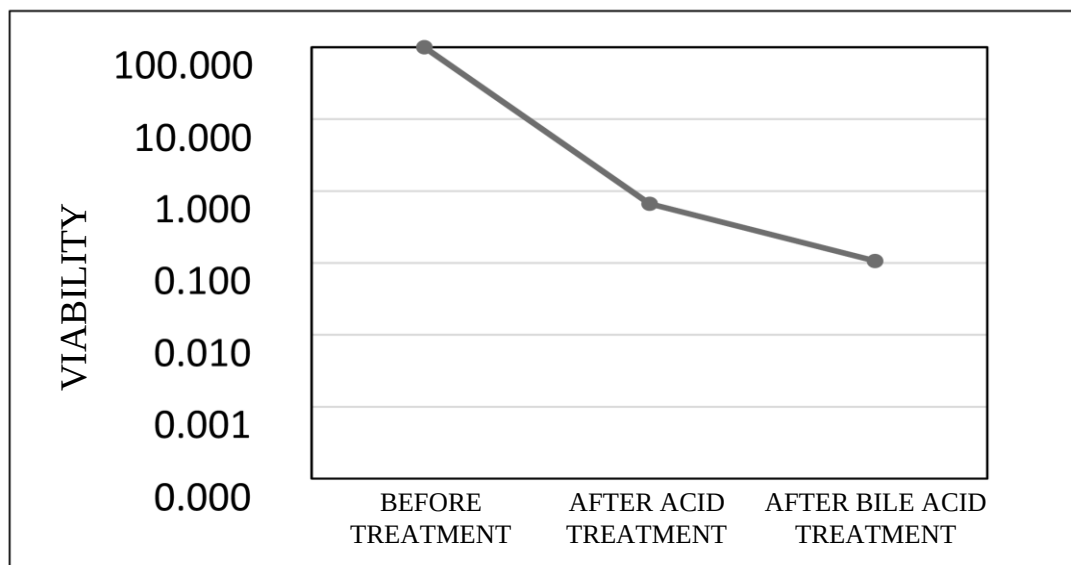
[Claim 7]

The food or beverage according to claim 6, which is a fermented milk food or beverage.

[Figure 1]



[Figure 2]



SEQUENCE LISTING

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